THE UNIVERSITY OF ALBERTA

OPTIMIZATION OF CHRONIC RECORDING FROM NERVE FIBERS AND ITS APPLICATIONS

BY

© JACK H. JHAMANDAS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

BIOPHYSICS

DEPARTMENT OF PHYSIOLOGY

EDMONTON, ALBERTA

FALL, 1976

IN MEMORY OF MY FATHER

ABSTRACT

A method is described for recording from peripheral nerves of the cat using silastic cuffs in which three or more electrodes have been inserted. A tripolar recording configuration between a central lead and the two end leads, which are connected together, provides good rejection of EMG from the hindlimb musculature. Further optimization of the neural signals is obtained by the use of transformers, impedance matching and filtering techniques. Simple models are constructed to predict and assess the performance of cuffs and transformers in recording neural activity. Consistent deviations from the theoretical models were observed at both high frequencies (reflecting primarily the changes in the tissue and fluid within the cuff) and at low frequencies (concerned mainly with electrode properties).

Recordings from intact nerves show that the peak-to-peak amplitude and latency of the compound action potentials remain stable over a period of several months. Voluntary neural activity is recorded from cats trained to walk on a treadmill. The behavioural signals are partitioned into motor and sensory components using cross-correlation techniques. An analog multiplier incorporated with a digital delay line was used for on-line cross-correlation. This permitted us to follow the dynamic response of afferent and efferent fibers. To aid in determining the quality of behavioural signals, spectral analysis of the recorded activity was carried out. Low frequency EMG potentials were distinguishable from the relatively high frequency neural components.

The methods are extended to record from severed nerves which

are placed in a cuff whose distal end has been sealed. It is possible to monitor the state of the axons by monitoring impedances of the nerve, the compound action potentials and voluntary activity at various distances from the sealed end. Sequential changes in the aforementioned parameters are observed and these changes closely parallel the degenerative and regenerative processes taking place in the nerve. Voluntary activity ceased to exist about a month after axotomy while it was still possible to record large compound action potentials by electrical stimulation of the nerve. The findings are discussed in light of past knowledge and present preoccupations of investigators in this area.

Some preliminary behavioural studies are described on the nature and role of reflexes in the distal hindlimb of the cat. In particular, a number of extensor reflex responses are observed by stimulating the tibial and the peroneal nerves. Perturbations introduced during the stance phase of the step cycle of a cat evoke a prolonged activation of the ipsilateral extensors. The role of the extensor reflexes in locomotion is discussed within the currently accepted hypothesis of centrally controlled rhythm generators.

Finally, the implications of the findings on intact and severed nerves are discussed as they pertain to the neural control of artificial limbs. The results from severed nerves without target organs seem negative but further studies in this area are needed before one can rule out the concept of neurally powered prostheses. The contribution of the methods described is particularly useful for behavioural studies.

ACKNOWLEDGEMENTS

I wish to extend my sincere appreciation to Dr. Richard B. Stein for introducing me to the field of neurophysiology and for his subsequent guidance of my research with patience and kindness. Nor has his contribution to my education been wholly academic, for he has exemplified to me the qualities of tolerance, humility and the ethic of hard work, and I am truly indebted to him for this.

To Dr. T.R. Nichols I extend many heartfelt thanks for having taught me physiology in a manner no text or course could match and for sharing with me as a friend the sometimes trying experience of graduate studies. Thanks, Dick.

I wish to thank Mr. Dean Charles for making the devices competently and for educating me in electronics and instrumentation. Thanks are also due to Dr. Lyle Davis for his help in implantation of the cuffs.

To Dr. J. Duysens I extend thanks for providing much useful discussion on behavioural studies. As well, I wish to thank Dr. P. Bawa for many helpful discussions during her stay here. Ms. V. Waivods provided much useful criticism in the preparation of this manuscript and I am grateful to her for this.

I wish to express my thanks also to Mr. Fred Loeffler and Mr. Ken Burt for their very competent and capable drafting and photography, and to Mrs. Ortella White for her efficient typing of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

For many years, neurophysiologists have attempted to analyze in some detail the functional architecture of behaviour in the mammalian nervous system. While similar efforts have met with considerable success in the invertebrate systems, the larger neuronal circuitry of vertebrates has until recently posed a formidable challenge. The lack of success in recording the activity of nerves and muscles in behaving animals is in part attributable to the absence of adequate recording methods until the last 20 years. The application of microelectrodes to the brains of unanaesthetized behaving animals (Hubel, 1957; Evarts, 1966) represents some of the first attempts in elucidating the neurophysiological correlates of behaviour in mammals. The use of microelectrodes has subsequently been extended to the peripheral nerves in man (Vallbo, 1971). These methods are not without their inherent drawbacks. Generally, the difficulties in using microelectrodes stem from their sensitivity to movement and also for the need for them to be carefully positioned at each recording session in order to ensure an adequate signal-to-noise ratio.

Intracortical microelectrodes, which have been the subject of considerable refinement, have now been used to record from individual neurons for a period of days and even months (Schmidt, 1975; Bak *et al.*, 1975). The quality of such recordings diminishes with time due to the growth of connective tissue and damage to adjacent cells by relative movement of the electrode and the tissue. Similar recordings from

peripheral nerves are even more difficult due to the absence of any fixation points for anchoring the microelectrode manipulators. The risk of infection from electrode penetrations is equally great in either of the above methods.

An alternate method to record chronically from the vertebrate periphery has been reported recently by Mannard *et al.* (1974). This group was able to record from amphibian nerves using regeneration electrode units, which consisted of holes in a wafer-thin non-conductor. The units were placed in the path of partially transected sciatic nerves of the amphibia. After some time chronic neural recordings were possible from both the motor and sensory fibers that had regenerated through the holes. Attempts to transpose the methodology to mammalian nerves have met with little success due to the small size of regenerated fibers and a greater proliferation of connective tissue (Stein *et al.*, 1975). A further constraint involved in this technique is that the new innervation may prove to be foreign to a particular end organ and therefore not reflect the true physiological pattern.

A more traditional method of neural recording and one known to physiologists for quite some time now involves dissection over a suitable length of a bundle of nerve fibers, which is then placed on metal hooks or electrodes in a non-conducting medium such as paraffin oil. The nerve bundle thereby provides a resistive pathways along which currents generated along the fibers can flow and be recorded as voltage differences between the metal hooks or versus a ground. The extracellular records obtained in this way are quite reproducible and stable over long periods of time. Most of this type of recording has been

confined to short-term acute preparations. It has been suggested that the coupling at the electrode-nerve interface could be made more permanent by wrapping a sleeve of non-conducting material (with internal electrode contacts) around the nerve. In this way, currents generated inside the cuff or sleeve would flow to ground via resistive paths afforded by the nerve filaments and be registered as voltage differences across the internal contacts. Although implantable electrodes have found their use in recording neural activity only recently, the idea of using them for chronic stimulation of the vertebrate periphery is by no means a novel one. For as early as 1933, Cannon has described the implantation of nerve cuffs around the autonomic nerves of cats in his efforts to study pain fibers. More recently, Brindley (1972) managed to implant Silicone rubber-Platinum wire electrode arrays around the dorsal and ventral roots of the baboon. However, both of these attempts were undertaken with the primary aim of stimulation rather than recording from the nerves and spinal roots, although Brindley did manage to show the morphological intactness of the encapsulated roots for periods up to 20 months.

The efforts of Frank (1968) heralded the advent of chronic neural recording from alert, freely-moving and behaving animals. In these experiments the finely dissected dorsal root filaments, with recording wires attached, were encapsulated with Silastic. Single unit activity was recorded from cats for up to 2 weeks. In the past 2 years, this concept has been extended to placing cuffs or tubes around the peripheral nerves (Hoffer *et al.*, 1974; Stein *et al.*, 1975; Schad and Seller, 1975; DeLuca and Gilmore, 1976). As previously described, the

method permits recording neural signals by insulating the nerves from body fluids and tissue. The adaptability of the method has been demonstrated unequivocably by its application to a number of diverse preparations. For example, Hoffer *et al.* recorded from the fine nerve filaments of the rabbit tenuissimus muscle, Stein *et al.* and DeLuca *et al.* have recorded gross activity from several different sized peripheral nerves of the cat and the rabbit respectively, while Schad and Seller have used the technique to record from autonomic nerves of the cat. The methodology in all these cases permitted neural activity to be monitored during normal behaviour and metabolism.

Our group (Stein *et al.*, 1975, 1976) has been able to show that, with proper precautions and care, it is possible to retrieve neural activity from cuffs (with varying numbers of internal electrode contacts) placed around intact peripheral nerves. With proper cuff geometry, the nerves have remained a viable source of neural signals for up to 6 months or more and auxilliary histological studies tend to confirm the intactness of these nerves. To date much of the effort has been focussed on attempts to optimize the quality and pattern of the neural signals, in the presence of the larger electromyographic (EMG) signals outside the cuff and interference from other environmental sources, such as 60 Hz fluorescent lights, radio waves, etc. The challenge to do this has been met with a combined use of particular electrode configurations, impedance matching and filtering techniques. The method has been perfected to the point where it is now possible to record neuronal signals from unrestrained, freely-walking cats on a treadmill in an unshielded room. The use of the cross-correlation technique has been developed to help in partitioning

the neural traffic into its afferent and efferent components.

This thesis attempts to recount the development and progress in our lab of the aforementioned optimization procedures over the past 18 months. Some insight is provided of the potential and usefulness of the methodology in both basic and clinical research. It is appropriate at this stage, therefore, to introduce some of the basic features of the cuff recording technique and to expound the principles underlying them.

A. CUFF ELECTRODES

The electrode, in its basic design, consists of one or more cuffs or tubes made of medical grade Silastic, each with a minimum of three internal recording contacts. The dimensions of the individual preformed Silastic tubes varied with individual implants, but a range in internal diameter (1.0 to 3.4 mm) and length (from 1.5 to 5.0 cm) was available. The contacts sewn into the individual cuffs consisted of single or multiple strand teflon-coated silver (Ag) or platinum-iridium (Pt-Ir) wire. In order to increase the area of contact for the pick up of neural signals, the wires were threaded back and forth within the cuff. The teflon-coated wires from each cuff were led to a twelve-pin integrated circuit socket, which in turn was fixed with epoxy in the center of a vitreous carbon connector (Biosnap). The Biosnap has been shown to be extremely biocompatible in its application to the skin of experimental animals and even human subjects. In our case, the Biosnap was sutured into the skin overlying the hip at the time of implantation. The skin in this area forms a tight seal around the connectors and

subsequent growth of the connective tissue through the holes of the flange further anchors the assembly. Fig. 1 illustrates one typical device containing three neural cuffs and an EMG probe, each with its complement of insulated cables, and a Biosnap. The primary requirements of such devices have been that they be flexible enough not to damage the nerves and at the same time possess enough tensile strength to withstand the strain over long periods of time in a freely-moving cat. Unduly stiff cables and connectors tend to distort the nerve geometry and this in turn leads to premature blockage of nerves. The attempts to fabricate devices with the optimum trade-offs are still continuing and recent progress in this area is documented in a recent publication (Stein *et al.*, 1976).

B. EMG REJECTION AND THE BALANCED CUFF CONFIGURATION

One of the major problems associated with recording neural activity is the contamination of the records by large amplitude, low frequency electromyographic (EMG) signals. The electrical activity of the muscles generated either during voluntary activity or by stimulation of peripheral nerves is usually large enough (10 to 30 mV) to swamp the relatively smaller neural potentials. Stein *et al.* (1975, 1976) have succeeded in overcoming this difficulty with the aid of a number of techniques. The single most important feature of these techniques is the use of a symmetrically balanced configuration, whereby the neural activity is recorded at the center of the cuff with respect to the two end contacts shorted together. Fig. 2 shows the essential features of this particular configuration. The principle underlying this is that when

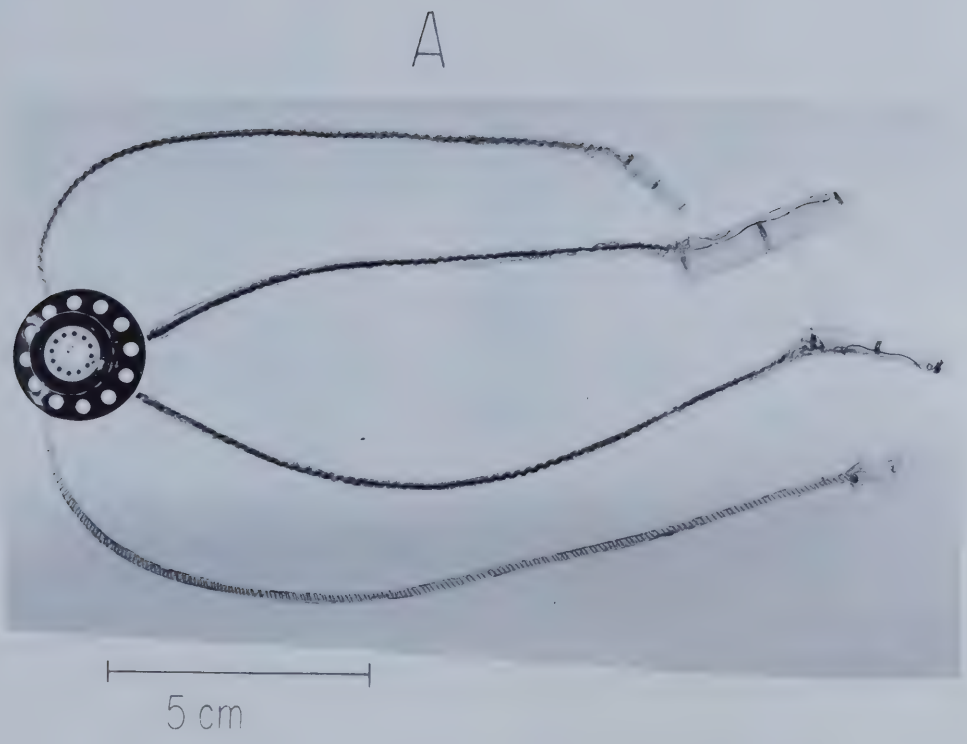


Fig. 1. The device shown here contains three neural recording cuffs and one EMG probe connected to a twelve-pin socket in a vitreous carbon button (Biosnap) which is used as a skin interface. Note the three electrode contacts in each neural cuff and also the coiled Pt-Ir wire in the cables.

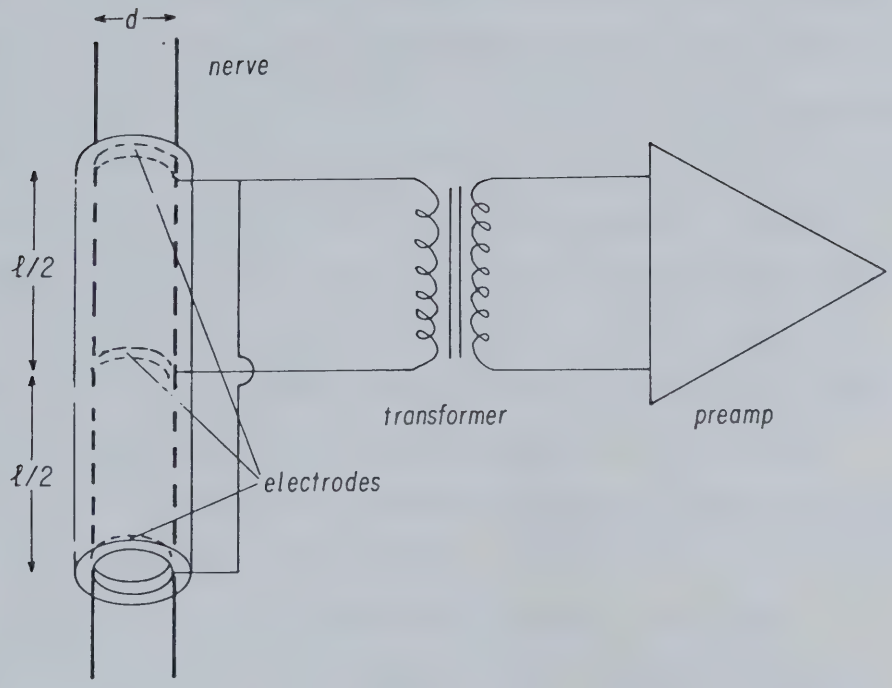


Fig. 2. Method for recording using an insulating cuff with three electrodes around a nerve. The balanced tripolar configuration shown here is effective in rejecting EMG signals by the virtue of its shorted end contacts. The principle underlying the use of this configuration in optimizing neural recording is discussed in the text.

the two ends of the cuff are shorted together, the large EMG signals will not be able to flow through the cuff in the absence of a potential gradient. Rather, these EMG signals will tend to flow around the cuff and the only signals propagating through the cuff will be the action potentials generated by the nerve within it. Fig. 3 serves to illustrate the concept. When the whole sciatic nerve of a cat is stimulated and the resultant activity recorded distally from a nerve segment enclosed in a non-conductor (mineral oil or cuff), two distinct potentials are evident (Fig. 3A). The early wave is the neural signal and the larger later component is the EMG. The more traditional common mode rejection (CMR) method used to reject EMG involves the placement of an indifferent electrode in the fluid near the recording electrode and feeding the EMG signals picked up by both electrodes into a differential amplifier, which would reject them. If the CMR technique is applied to the signal (Fig. 3A), the EMG is still large enough to swamp the neural component (Fig. 3B). However, if the tripolar balanced configuration in Fig. 2 is used, the neural-to-EMG ratio is greatly enhanced (Fig. 3C). The residual EMG accompanying the neural signal is due to a number of factors.

For a perfect short between ends of the cuff, the contact impedance of the electrodes with the tissue should ideally be zero. However, at frequencies of interest, the non-zero impedance of the electrode (see Chapter 2) prevents an effective short, thereby allowing low frequency signals (mainly EMG) to leak into the cuff.

Another major factor contributing to the presence of the residual EMG is the potential gradient set up in the radial direction by the currents generated from the nerve fibers within the cuff. Hoffer

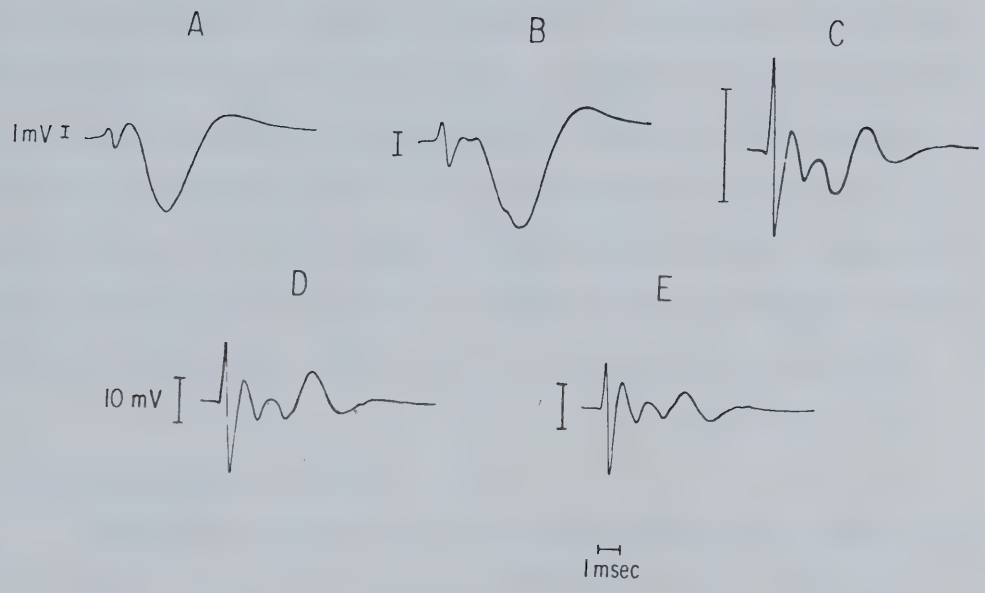


Fig. 3. Compound action potential recorded by an electrode at the center of a cuff 4 cm in length around. The ventral roots supplying this were stimulated and recording was carried out with respect to A) an electrode some distance away (about 20 cm), B) an electrode outside the cuff, C) electrodes at the two inside edges of the cuff which were connected together (see Fig. 2). In D) and E) the configuration was the same as in C) but a transformer was used to amplify the signal in D) and further filtering was done in E) to improve the neural signal (early, fast waves) with respect to the slower EMG waves. The vertical bar represents 1 mV for each part in the top half of this Fig. and 10 mV in the bottom half. The time scale remains the same for the whole Fig.

(1975) has calculated the contribution of the large transverse resistivity (ρ_t) to the development of such voltage gradients and from his calculations it would appear that this potential radially is substantial (about 98 μV at a distance 30 μm from the nerve fiber of conduction velocity 64 m/sec).

It is important that the geometric symmetry of the tripolar recording configuration (shown in Fig. 2) be preserved, for a shift in the positioning of the indifferent end electrodes or a gross impedance mismatch between the two is liable to affect adversely the magnitude of the neural signal and the ability of the configuration to reject EMG.

C. AMPLIFICATION AND FILTERING

Additional features have been incorporated in this technique to further enhance the quality of neural activity recorded. In Fig. 3D, a high gain Hammond transformer (585-D) is placed between the electrodes and the preamplifier. As is evident, the neural signal has been amplified over ten-fold and further filtering is added. The properties and the role of the transformers is dealt with more fully in Chapter 2. From spectral estimates the frequency of the neural signals is determined to be in the order of 1 to 2 KHz, as opposed to that of the EMG, which is about 100 to 300 Hz. Some form of high pass filtering therefore is quite desirable and Fig. 3E shows the effect of incorporating a first-order high pass filter (half power point of 300 Hz).

The principles outlined above are not only applicable to the compound action potential records, but also to the optimization and analysis of behavioural data comprising of both voluntary and evoked reflex activity.

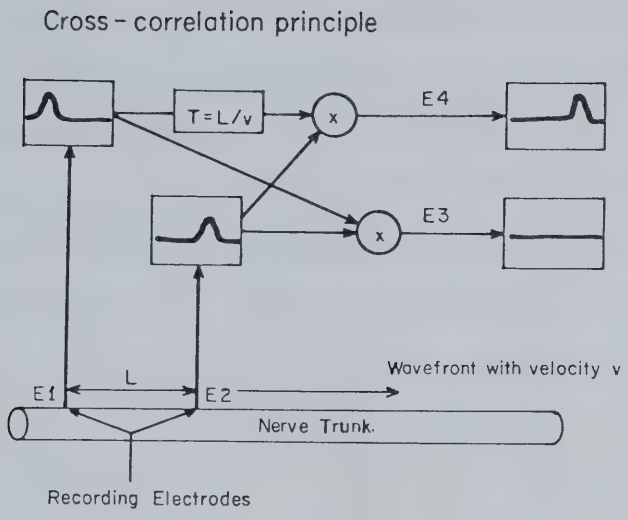
D. THE PARTITIONING OF NEURAL ACTIVITY

The ability to record chronically from peripheral nerves allows us to monitor the total neural traffic involved in specific behavioural tasks performed by freely-moving animals. Recently there have been a number of reports using time convolution techniques to sort out the gross peripheral nerve activity (Mann, 1973; Heetderks and Williams, 1975). Methods of optimal linear filtering have been developed and successfully applied to invertebrate systems (Roberts and Hartline, 1975). These techniques permit the partitioning of total nerve activity into different afferent and efferent components on the basis of direction of travel, conduction velocity and waveforms.

The technique of cross-correlation, although known for some time now, has only recently found widespread use in the segregation of axonal conduction delay groups. The mathematical basis of the operation involves a point-by-point multiplication of two analog signal records at discrete points in time and a summation of the products. The multiplication is repeated for a series of time shifts where one waveform is fixed and the other is shifted by a time τ. The summation of products at each point in time plotted versus the discrete time shifts gives a cross-correlation. Eqn. 1.1 and Fig. 4A summarize the essential features of the operation.

$$R_{xy}(\tau) = \lim_{T\to\infty} \frac{1}{T} \int_0^T x(t)y(t+\tau)dt \qquad \text{Eqn. 1.1.}$$

The above operation can be performed on a previously programmed Lab 8 computer system (Digital Equipment Co.) by playing back the data recorded



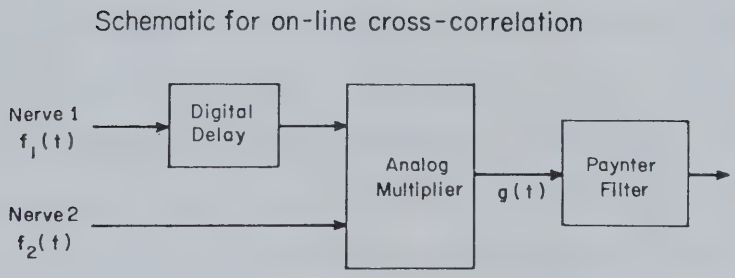


Fig. 4. a) Diagram summarizing the cross-correlation principle.
b) On-line cross-correlation between two nerves. For cross-correlating the motor activity the digital delay line is inserted on the more proximal of the two nerves. For sensory activity the delay line is switched to the the other nerve.

on an FM tape recorder (Hewlett-Packard 3960). Alternately, to obtain specific modulation of motor, sensory or cutaneous activity, a scheme such as shown in Fig. 4B was used. A digital delay would be incorporated into one of the channels (of neural activity) to the analog multiplier. This would delay the signal arriving at Nerve 1 by a fixed time τ. The analog multiplier would then perform a point-by-point multiplication operation on the signals and sum or integrate the products. The output of the analog multiplier would then be the cross-correlation of the two signals:

$$g(t) = f_1(t+\tau)f_2(t) \qquad \text{Eqn. 1.2.}$$

The output may then be filtered and displayed as required. The additional feature afforded by this method is that a number of delays (depending on the changes in waveform and conduction velocity with time) could be used for afferent and efferent activity. A measure of the correlated activity as a function of delays is then possible. Details of this technique as applied to the neural activity recorded during treadmill walking of a cat are elaborated in Chapters 2 and 3. The design and construction of the multiplier and the appropriate filtering are also discussed.

It is hoped that the stage has now been set for the reader to follow the changes in pattern of neural activity as they apply to intact and severed nerves. Chapter 2 deals with the long-term recording from intact peripheral nerves with particular reference to the use of transformers and impedance measurements as aids in the optimization

procedures. Long-term electrical recording from nerves following axotomy is the subject of Chapter 3. The methodology described for intact nerves is extended to severed nerves in cuffs whose distal end has been sealed. Much of the concern centers around the fate of the axotomized nerves and the sequential changes observed in measurements of impedance, compound action potential amplitudes, conduction velocities and voluntary activity. The results as they pertain to amputee-like situations are discussed in light of past and present clinical knowledge. More general discussion in terms of the trophic interactions between the nerve and its target organ is included. Chapter 4 is devoted to the application of the methods described earlier to specific behavioural and reflex studies in locomotion of cats. These studies, although of a preliminary nature, are indicative of the vast potential of the technique in confirming or rejecting the accumulation of information from behavioural work in thalamic or mesencephalic preparations. In the final chapter, some of the implications of these studies are discussed as they apply to the neural control of artificial limbs and the study of the neurophysiological basis for behaviour in animals and man.

CHAPTER 2

ON RECORDING CHRONICALLY FROM INTACT PERIPHERAL NERVES

The many facets of the methodology as they pertain to recording from intact nerves are the subject of this Chapter. Some of these have already been dealt with briefly (Chapter 1) and will placed in their proper perspective. Others, such as the use of transformers, impedance measurements and the general characteristics of the neural activity, are expounded on in some detail. As well as describing the experimental aspects of the optimization procedures, certain theoretical models are constructed to predict and fit the data. The incorporation of such theoretical models into the study allows one to assess beforehand the performance of discrete individual components such as the cuff-electrodes, transformers or a combination of the two. This information can then be transposed to a given experimental situation so as to optimize the retrieval of neural signals. In the interests of clarity and brevity, no attempt is made to segregate the results from their interpretation or discussion, but each aspect is dealt with *in toto*.

A. IMPLANTATION OF CUFFS

The surgical implanting procedure was carried out under full aseptic conditions. The cats were anaesthetized with a 30 mg/kg dose of Nembutal (Abbott Lab.) administered intraperitoneally. The hindlimb to be used for implantation was shaved and prepped with Betadine solution. A midline incision was made in the lateral region of the thigh for access to the sciatic nerve. The incision was deepened through the

subdermal fascia and the nerve, with its blood supply intact, carefully dissected free of the surrounding tissue. The procedure was repeated for the more distal branches of the sciatic nerve (the lateral gastrocnemius-soleus, the common peroneal, the sural and the tibial nerves). The skin was freed from the subdermal tissue in the hip region for installation of the percutaneous connector (Biosnap) and a channel dissected under the skin from this site to the nerve. The nerve was then gently slipped into the appropriate cuff with the aid of forceps and the cuff closed with interrupted silk sutures (4-0) placed every 2-3 mm. Silastic was sometimes applied to reinforce the seal in order to prevent the seepage of fluids and the growth of connective tissue through the slit in the cuff. In most instances better EMG rejection was obtained where a combination of the mechanical and chemical seals was used.

In some experiments EMG electrodes of the type shown in Fig. 1 were sutured onto flexor (anterior tibialis) or extensor (gastrocnemius-soleus) muscles of the distal hindlimb. After installation of the electrodes, the flexibility of the cuffs and cables was adequately tested by manipulation of the leg. The Biosnap was positioned through a small incision in the skin of the hip area and a purse-string suture applied to hold it in place (3-0 chronic). The muscle fascia and the skin edges were approximated with 3-0 chronic to achieve closure of the incisions. If necessary, additional Nembutal was administered intravenously during the operation and the entire surgery took about 2-3 hours to complete.

B. RECORDING METHODS

(i) Under Anaesthesia

Recordings were made immediately following surgery, while the cat was still under Nembutal anaesthesia. At subsequent post-operative recording sessions, the cats were anaesthetized with Halothane (Halocarbon Ltd., Ont.) from a regulator (Fluotec Mark 2, Cyprane Ltd.). A 12-pin plug was inserted into the socket of the Biosnap to negotiate contact with each individual lead from the cuffs. The plug was connected to a switching box via a flexible, shielded cable. The switching box was so designed that any lead (or combination of leads) could be connected to a stimulator or conventional preamplifier (Grass P5), either directly or through a transformer coupling. Conventionally, pins marked 1 and 12 on the box were used as ground leads and the remaining ten appropriately distributed amongst the various cuffs comprising the device.

Routine measurements of impedances provided a very sensitive and remarkably effective method of monitoring the behaviour of a nerve in a cuff over time. The electrode impedance was measured with an impedance meter (Hewlett-Packard, Model 4800A), which injected sinusoidal currents between the appropriate contacts and displayed the vector impedance values after accounting for the various parameters, i.e., the voltage developed between contacts, the magnitude of current injected, and the geometry of the electrodes. At the start of each recording session, the impedance of all the leads versus ground was checked at 1 KHz. This helped to establish any lack of continuity due to wire breakage. The impedance of particular electrode configurations (tripolar or bipolar) as a function of frequency was also measured and the relevance of this

procedure is dealt with later (see Impedances).

In order to ascertain the capability of a particular nerve to conduct impulses over time, the compound action potentials were recorded by electrically stimulating the nerves. For example, the sciatic nerve was stimulated maximally through the electrode contacts in the cuff around it and the resulting compound action potential was recorded from the distally-located nerve cuffs (LGS, sural, common peroneal or tibial). The stimulating and recording leads were reversed to back-stimulate and record the neural potential on the sciatic nerve. All signals were observed on a storage oscilloscope and either photographed directly or recorded on an FM tape recorder (Hewlett-Packard, Model 3960). As the animals were allowed to recover from the anaesthesia, natural stimulation was applied to the appropriate sensory fields for each of the peripheral nerves and the evoked neural activity recorded. Off-line processing of all data was done using a preprogrammed Digital Equipment Lab-8 computer.

(ii) Voluntary or Behavioural Activity

Cats whose nerves showed promising signs of yielding behavioural data were subjected to a fairly intensive period of training in treadmill walking. Naive cats were induced to walk on a motor-driven treadmill for a food reward. Usually the cats became adept at voluntary locomotion after half a dozen trial sessions, but in cases where initiative was lacking, reinforcement to the food reward was provided in the form of an air stream to the rear of the animals.

After the training was complete, the freely-walking cats were recorded from periodically. The Biosnap was interfaced with the recording

equipment via a flexible cable running through a harness attachment. This type of an arrangement permitted considerable freedom of movement for the cats and at the same time guarded against undue strain being applied to the Biosnap. Fig. 5 illustrates the recording set-up used. We recorded the neural activity from each of the cuffs using the appropriate transformers, amplification and filtering (300-10 KHz frequency cuts). Concurrently, EMG activity from the triceps surae (ankle extensors) or anterior tibialis (ankle flexor) was monitored by recording either differentially outside the cuff or with a separate EMG probe (see Fig. 1) on the muscle of interest.

A maximum of four channels was available to accept input from the various electrode configurations, which could be cross-connected on the front panel of the switching box. Grass P-15B A.C. preamplifiers were used to amplify the neural signals by a factor of 1000 and the larger EMG potentials by 100. The output of the four differential amplifiers was displayed on an oscilloscope (Tektronix, Model 5403) and recorded in parallel channels on an FM tape recorder (set at maximum gain of about 6). The output of the tape recorder was checked from time to time to prevent saturation at high gains. Voice commentary was interjected into the records to facilitate the eventual retrieval of information.

During the recording sessions no special precautions were taken to shield either the motor of the treadmill or other sources of interference in the environment (i.e., fluorescent lights, mains cords, etc.). 60 Hz interference from the power outlets sometimes contaminated our records, but proper grounding usually rectified the situation.

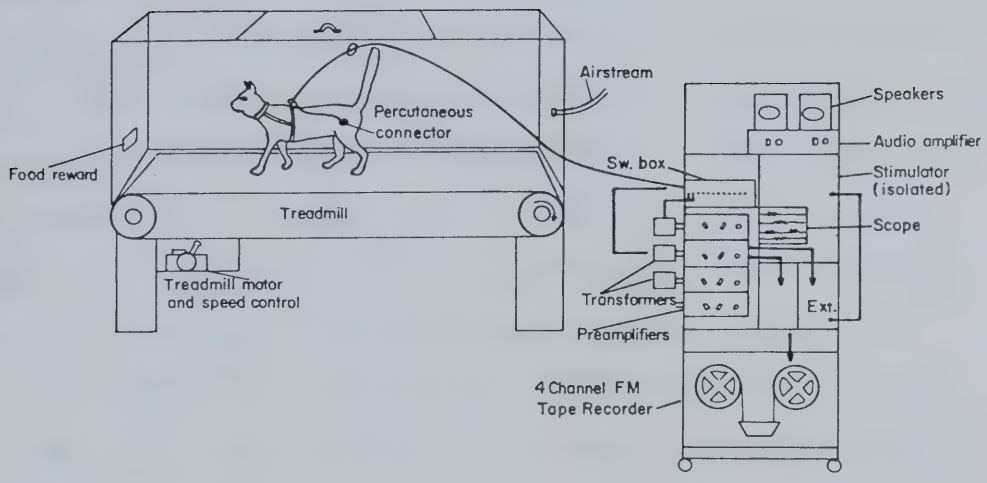


Fig. 5. The set-up for recording voluntary activity. The cat was induced to walk on the treadmill in return for a food reward. Neural and EMG activity was recorded on a maximum of four channels. For behavioural studies (see Chapter 4) stimuli were delivered from the isolation unit.

C. AMPLIFICATION AND FILTERING

(i) Transformers

The high gain transformers (Hammond 585D and 585F) used in the present experimental study have several important functions. Most physiological amplifiers have high input impedance ($\geq$ 10 M) and approach the minimum noise level theoretically possible (the so-called Johnson or Thermal noise) with a high impedance source. However, these preamplifiers are not optimized for low impedance sources such as neural signals. By using a transformer with a low impedance primary and a high impedance secondary, the impedance can be matched to that which the preamplifier handles best. Previously (Buchthal & Rosenfalck, 1966; Stein *et al.*, 1975) it was argued that the Johnson noise level increases only as the square root of the source impedance, so an increase in signal-to-noise ratio equal to the square root of turns ratio is possible. Recently it was pointed out (R. Scott, personal communication) and confirmed independently by myself that a transformer changes voltage by a factor equal to the turns ratio and impedance by a factor of turns ratio squared. Therefore, the source signal-to-noise ratio cannot be improved by adding a transformer, and the fact that we do observe an enhanced signal-to-noise ratio must be attributable to the particular low-noise characteristics of the amplifiers used.

The transformers also provide some extremely useful filtering characteristics. This statement requires some explanation since normally preamplifiers are designed to operate with a wide range of frequency cuts and high input impedance, so as to distort the biological signal as little as possible. The input stage is often directly coupled to the preparation,

since capacitative coupling could be detrimental to the common-mode rejection of the preamplifier and may increase the noise level. Filtering is done at a later, less sensitive point in the circuit. However, with direct coupling, transients can cause blocking of the preamplifier for a period of time and low-frequency movement artefacts are also troublesome. The filtering aspect of the transformer can help alleviate these difficulties with low-frequency components.

Fig. 6 shows the impedance of typical cuff electrodes 1 month after implantation. There is a fairly constant resistance at high frequencies which is mainly due to the resistance of fluid filling the cuffs and their fibers. The resistance of a cylinder of saline of resistivity ρ, length l, and diameter d is (Mannard *et al.*, 1974)

$$R = \frac{\rho l}{\pi d^2} \qquad \text{Eqn. 2.1.}$$

The sciatic and lateral gastrocnemius-soleus (LGS) nerve cuffs shown in Fig. 6 had lengths l = 2.0 cm and 1.2 cms and diameters d = 0.34 cm and 0.15 cm respectively. If the effective resistivity were ρ = 200 Ω-cm (Ruch & Patton, 1965), one would expect resistances of about R = 1.1 KΩ and 3.5 KΩ from Eqn. 2.1, in reasonable agreement with the observed values. The seemingly higher values in Fig. 6 may be explained by the increased effective resistivity due to the proliferation of connective tissue into the extracellular space. At low to intermediate frequencies the impedance varies inversely with frequency. This is due to the capacitance of the electrode-saline interface (Robinson, 1968; Pollak, 1974c) and is dealt with later under Impedances.

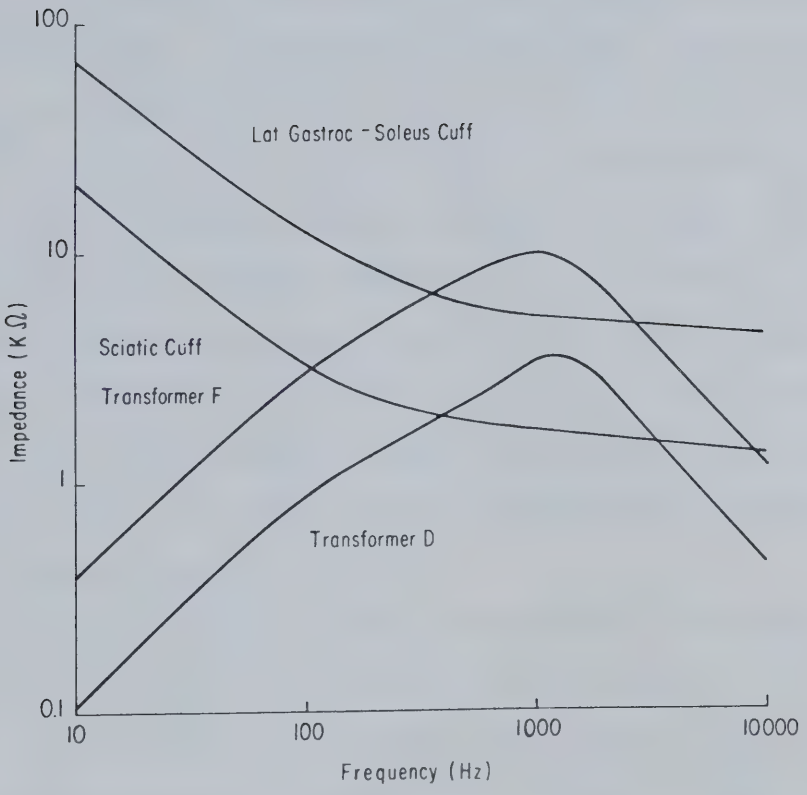


Fig. 6. Magnitude of impedances as a function of frequency measured for two different cuffs: one around the whole sciatic nerve and a second around the branch to lateral gastrocnemius and soleus muscles. Also shown are the impedances of two transformers (Hammond 585D and 585F) which were tested in the circuit shown in Fig. 2 for use with these cuffs. The ratio of turns on the secondary to that of the primary is 25.2 for the 585D transformer and 12.6 for the 585F transformer.

Finally, Fig. 6 shows the impedance of two transformers as a function of frequency. Note that the impedances are highest at frequencies just above 1 KHz, whereas at low frequencies the impedances are much lower than that of electrodes. Since the capacitance of the electrode and the transformer are effectively in series, only a small fraction of the nerve voltage will be dropped across the transformers at low frequencies. This will not be true at frequencies where the impedance is higher, so the transformer serves as a band-pass filter. This is shown quantitatively in Fig. 7 by plotting the total amplification of the electrodes plus transformer as a function of frequency. The response is best at frequencies near 1 KHz, which is well within the neural domain and low-frequency signals such as 60 Hz interference, movement artefacts and components of EMG are strongly rejected.

The frequency response curve was determined by stimulating through one cuff (e.g., LGS) and recording the evoked potential in a second cuff (e.g., sciatic). The responses were averaged and the fast Fourier transforms were used to determine the magnitude of components at each frequency 1) when the electrode was directly connected to the preamplifier, and 2) when a transformer was inserted in between the two as shown in Fig. 2. The ratio of the magnitude of the components recorded with and without various transformers as a function of frequency was determined and is shown in Fig. 7. This ratio is a measure of the extent to which a given frequency component of a signal will be amplified (ratio > 1) or attenuated (ratio < 1).

From Fig. 7 it is evident that the transformers behave differently with the two nerves. For a nerve with an impedance as high as that of the

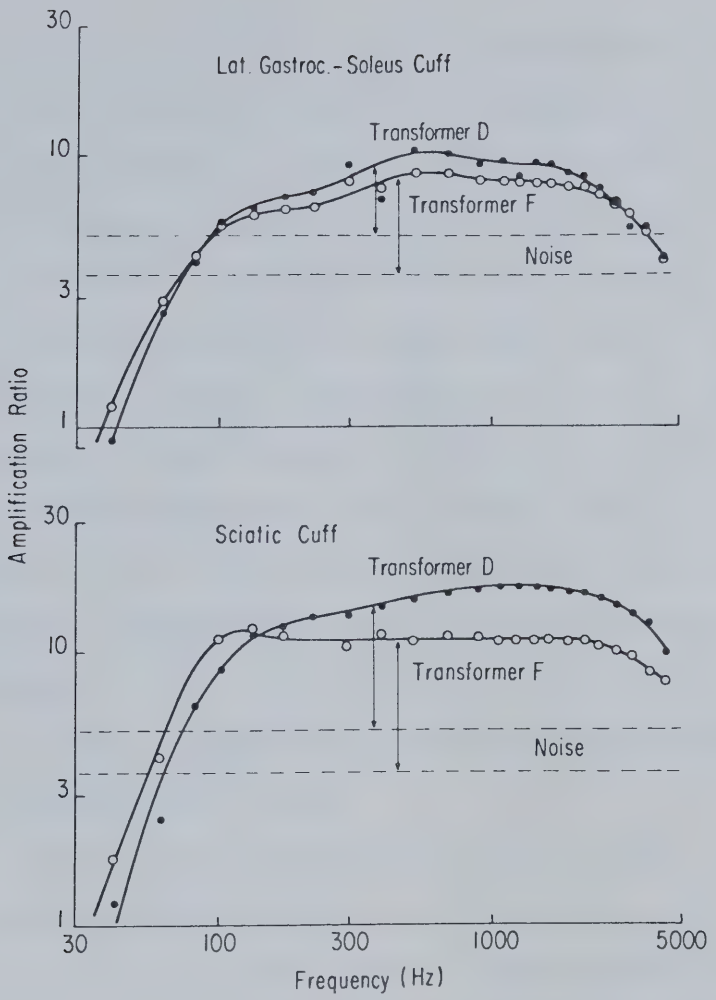


Fig. 7. Amplification of neural signals (● and O) and noise (interrupted lines) by two transformers (Hammond 585D and 585F). The data points for each transformer have been fitted by eye with a smooth curve for clarity. The vertical lines connecting the signal level to the noise level indicate the improvement in the signal-to-noise ratio at one frequency. As discussed in the text, transformer F was more suitable for use with the lateral gastrocnemius-soleus nerve, while transformer D was better with the sciatic cuff.

lateral gastrocnemius-soleus, there is little difference in the amplification of the signal by the two transformers, but the noise level is substantially greater with the D variety. Thus, transformer F provides the best signal-to-noise ratio and the signal-to-noise ratio is substantially improved on by this transformer in the range 100-4000 Hz. However, with a larger nerve such as the sciatic, and hence a lower impedance, transformer D provides a greater amplification of the neural components and better rejection of lower frequency signals such as the EMG. Thus, transformer D is preferable for use with the sciatic nerve. It is clear, therefore, that in order to optimize the transmission of desired signals and reject unwanted frequency components, one has to carefully match the recording apparatus to a given nerve. This can be particularly useful if a method or a model is available to give the pertinent information prior to the implantation of a device.

Equivalent circuits have been derived for the small signal impedance of metal bioelectrodes (Pollak, 1974a; Robinson, 1968). Using the pertinent data from Fig. 6, the impedance characteristics of the cuff electrode-tissue interface have been modelled using a simple RC network. The impedance-frequency responses of transformers (Fig. 6) have similarly been modelled using an RLC parallel resonant circuit (Skilling, 1957; Diefenderfer, 1972). The overall impedance of the cuff electrode and the transformer is therefore a result of the combined action of the equivalent network elements. Using both current and voltage sources, the impedance-frequency characteristics of the whole system have been analyzed and simulated. The use of a current source in the analysis is much more valid in that it approximates more closely the physiological

behaviour of a nerve as a current rather than as a voltage source. The mathematical basis for the analysis is outlined in Appendix I. The response of the model network, in terms of amplifying or attenuating signals of different frequencies, is strikingly similar to that obtained under experimental conditions. Fig. 8 shows the predicted response of a model network constructed from the parameters of a tibial nerve cuff and transformer F. It also displays the behaviour of a cuff-electrode-transformer F combination 6 weeks after implantation. The process by which the experimental response was arrived at has already been described earlier for the sciatic and lateral gastrocnemius-soleus nerves. The remarkable agreement between the theoretical and the experimental curves, particularly in the frequency domain of neural signals, underlines the usefulness of models is ascertaining the optimum transmission of the desired signals via appropriate cuff-transformer combinations.

(ii) Impedance Measurements

The relevance and importance of routine impedance measurements is fairly obvious from the previous sections of this Chapter. In the pioneering stages of this project, silver (Ag) wires were used in the construction of the cuff electrodes. However, after a month or two of implantation, the silver electrodes became quite brittle and often fractured during the course of post-mortem examination. In all, 7 out of 25 chronically implanted cuff electrodes were removed due to silver lead breakage. To make the devices more robust in character, platinum-iridium (Pt-Ir) was used in all subsequent cuffs and the use of silver discontinued except for acute experiments.

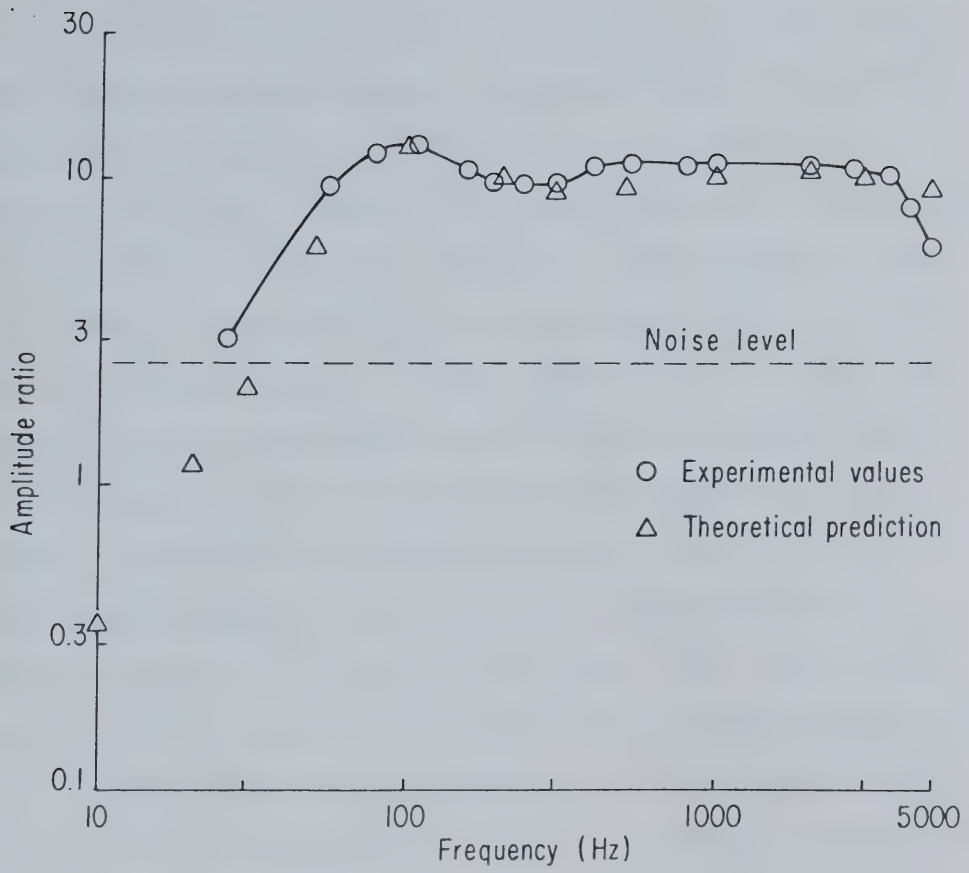


Fig. 8. Amplification of neural signals using a 585F transformer with a cuff around the tibial nerve. The theoretically predicted values (Δ) were arrived at using the impedance specifications of the cuff-transformer combination (see Appendix I) and the analysis was carried out before the implantation of the cuff around the nerve. The experimental values (O) fitted with a solid line were obtained in the same manner as those for Fig. 7 (see text). Note the close agreement between the preoperative theoretical prediction and the experimental data of 6 weeks post-implantation in the frequency region of interest (100 Hz - 1000 KHz).

In making the transition from silver to platinum-iridium, several differences were observed in impedance changes of electrodes over time. Since these changes are eventually reflected in the quality of the signals obtained, it is worth trying to understand the physical basis underlying them in other than purely empirical terms. Fig. 9 shows the impedance of silver and platinum-iridium wires 3-4 weeks after being implanted in a cuff around the sciatic nerve. The internal diameter of both cuffs was 3.4 mm and the distance between the electrodes was 3 cm. Recordings were made between the central electrode and the two shorted end electrodes, as described previously (see Recording Methods). Also shown is the impedance of the RC circuit given by the solid lines in the inset. This is the simplest circuit which can approximate the behaviour of the cuff and electrodes.

The agreement between the predictions and experimental data in Fig. 9 is by no means perfect. Consistent deviations were observed at both low and high frequencies, and the reason for these will be considered later in this section. However, for the moment, it will be useful to consider the physical basis of this simple model. The electrode-fluid or electrode-tissue interface forms a double layer which acts as a capacitative barrier to current flow. This capacitor C is particularly important at low frequencies.

The resistance R in the circuit is that of the tissue and fluid filling the cuff and it is across this resistance that the neural currents develop the voltage which is represented as input to the recording electrodes. The resistance will be particularly important at high frequencies, and indeed one can calculate the effective resistivity

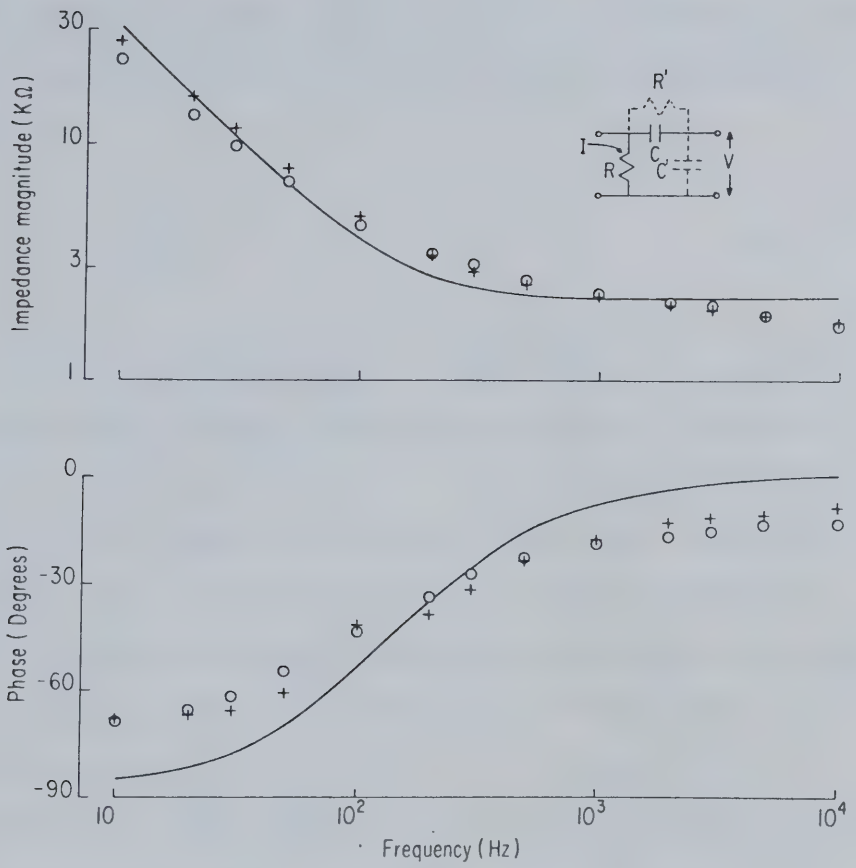


Fig. 9. Magnitude and phase of the impedance recorded from Ag (+) and Pt-Ir electrodes (0) several weeks after being implanted in a cat. The solid lines in the plot are predictions from a simple RC circuit shown in the inset with R = 2.2 KΩ and C = 0.51 μf. Note that the experimental points deviate somewhat from the predictions. The interpretation of these data, the RC circuit and the extra elements R' and C' are contained in the section on Impedances.

of the tissue, assuming it is the sole contributing factor to the high-frequency impedance. As mentioned earlier (under Transformers), one can obtain the expected impedance using Eqn. 2.1, or alternatively, knowing resistance R, the equation 2.1 can be inverted to give the apparent resistivity

$$\rho = \pi d^2 R/l \qquad \text{Eqn. 2.2}$$

where d = diameter of the cuff and l = length of the cuff. If these dimensions are given in cm and the resistance in Ω, the dimensions of the resistivity will be given in Ω-cm. Fig. 10 shows the impedance of cuffs around various nerves as a function of time after implantation at a low frequency (10 Hz) and a relatively high frequency (1000 Hz). Note that the values after implantation were considerably higher than the control values measured in saline (S) before implantation. The apparent resistivity calculated from Eqn. 2.2 indicates that the resistivity increased from about 80 Ω-cm in saline to slightly more than double that value in the body of the animal. This is because of the greater impedance of the tissue and to a lesser extent the more complete sealing of the cuff during implantation. The effective resistivity continued to increase for a few weeks after implantation to values of 200-250 Ω-cm using either silver or platinum-iridium electrodes. This increase is presumably due to the replacement of the fluid within the cuff by connective tissue, rather than to any electrode properties.

There were also changes in the low-frequency impedance over time which are shown in the upper part of Fig. 10. These did vary

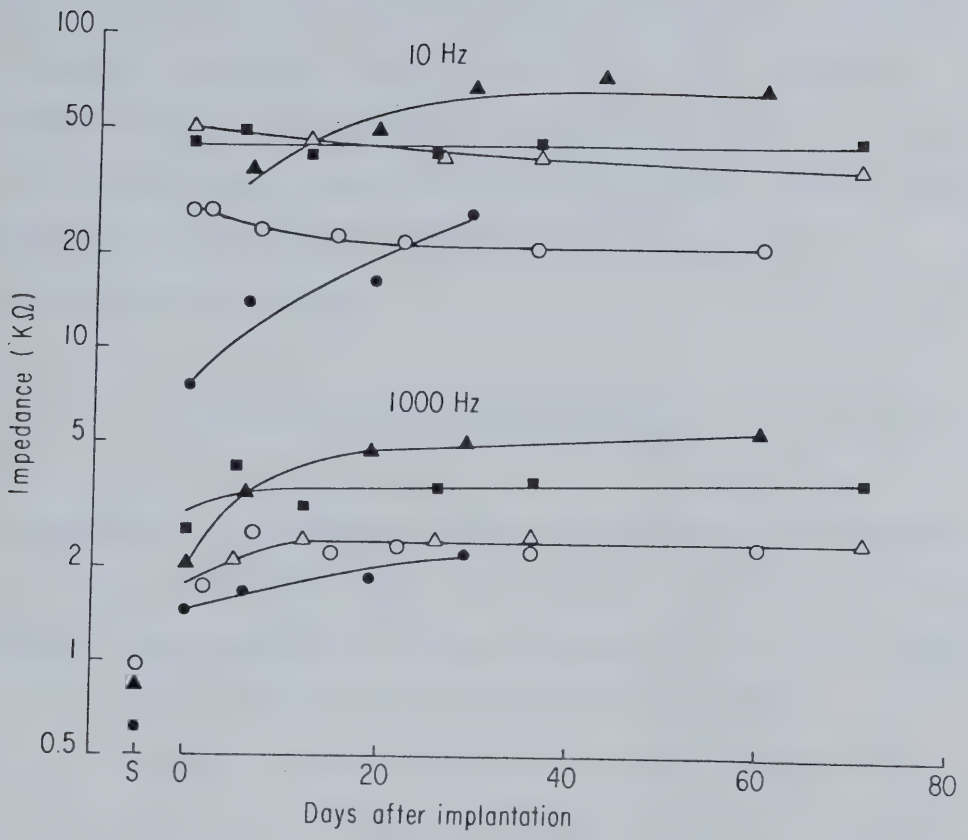


Fig. 10. Magnitude of the impedance for electrodes at a low frequency (10 Hz) and a relatively high frequency (1000 Hz) as a function of time after implanting the devices. The impedance of some electrodes was measured prior to implantation in physiological saline (s) at 1000 Hz. The impedances were measured between an electrode in the center of the cuff and the electrodes at the two ends of the cuff. The metals and nerves used were: ■ Ag, tibial nerve; ● Ag, sciatic nerve; ▲ Ag, lateral gastrocnemius-soleus nerve; O and Δ Pt-Ir, sciatic nerves. The impedance of all leads tended to increase at high frequencies due to growth of connective tissue into the cuff. At low frequencies the two metals behaved differently. Further discussion in the text under Impedances.

between the two types of electrodes used and thus showed dependency on electrode properties. The impedance of the silver electrodes remained constant or increased with time, perhaps due to the formation of a thicker silver-silver chloride layer with time. A thicker layer would increase the impedance by decreasing the capacitance (and increasing the resistance).

$$Z_c \text{ (capacitative impedance)} = \frac{1}{j\omega C} \qquad \text{Eqn. 2.3.}$$

The impedance of the platinum-iridium electrodes decreased about 25% slowly over a couple of months and then stabilized. The cause of this decline is open to question, but could be due to the initially smooth surface of the electrode becoming roughened or platinized.

Although the model in Fig. 9 serves to explain the basic principles underlying the observed impedance changes and is useful in designing devices for chronic use, certain deviations from the actual behaviour of electrodes were consistently in evidence. The circuit diagram of Fig. 9 assumes that the electrode can be considered as a resistance due to the fluid and tissue filling the cuff and a capacitance due to the double layer which forms at the electrode-fluid or electrode-tissue interface. This double layer has been extensively studied by physical chemists (see for example Bockris and Reddy, 1970). If the electrode is non-polarizable (e.g., if the silver wires were chlorided to provide a good Ag-AgCl surface), then the resistance R' shown by the dotted lines in Fig. 9 would have to be considered. It would limit the impedance to some finite value at low frequencies. An

unchlorided silver wire or a platinum-iridium wire is a polarizable electrode which does not pass DC currents (i.e., its impedance approaches ∞ near 0 Hz). Over the frequency range of interest to us (10-10,000 Hz), we rarely saw any evidence of the resistor R' shown by the dotted line, and it will therefore be ignored.

The voltage developed will therefore be connected to the recording equipment capacitatively. There will also be some stray capacity C' between the leads and ground (the shield on the cable to the recording equipment was generally connected to ground). This stray capacity will produce a decrease in impedance and a loss of signal amplitude at sufficiently high frequencies. There was usually some indication of a downward trend in impedance near 10 KHz attributable to this stray capacity, but its effect was small, as can be observed from Fig. 9.

Some other deviations from the model circuit were consistently observed. Firstly, rather than approaching a phase lag of 90° at lo_w frequencies, as expected for a simple capacitor, the phase lags approached 70° in Fig. 9 and 80° in other instances. Consistent with this was the fact that the slope of the gain curve was less than 1 (i.e., the impedance decreases as a fractional power of frequency). In Fig. 6 the slope is about 0.85 and in most other cases ranged from 0.75 to 0.85. This discrepancy has been noted previously (Robinson, 1968; Pollak, 1974c) and has been discussed in terms of the physical chemistry of the electrode-fluid interface. Nonetheless, the low-frequency impedance with time (Fig. 10) reflected changes in electrode properties. Secondly, the phase at high frequencies did not approach 0 as from the model, and

the magnitude of the impedance was not constant at high frequencies. Part of this deviation may be due to stray capacity, as indicated above, but some is also attributable to the properties of the diffusion layer outside the electrode (Pollak, 1974b). The effect of this layer is to produce the so-called Warburg impedance which decreases with frequency according to a square root relation (a slope of $-\frac{1}{2}$ on a log-log plot such as Fig. 9). The dependence of impedance on frequency was never this steep at high frequencies. Thus, electrode properties may add somewhat to the impedance and stray capacity may detract from it at high frequencies, but the major contributor to the impedance at high frequencies was due to the fluid and tissue filling the cuff. This conclusion was strengthened by calculating the effective resistivity of the fluid or tissue filling the cuff, assuming this resistivity was the sole contributor to the impedance at high frequencies. The calculated values (150-250 Ω-cm) agreed with previous independent measurements (Tasaki, 1964; Ruch and Patton, 1965).

D. CHARACTERISTICS OF RECORDED NEURAL ACTIVITY

(i) Compound Action Potentials: Their Form, Amplitude and Time Course

When a length of nerve is picked out of saline and insulated from the body fluids either in a mineral bath or a cuff, the form and amplitude of the action potential registered will be dependent upon the placement of electrodes (Stein and Pearson, 1971). A triphasic action potential will be recorded if the tripolar configuration shown in Fig. 2 (see Introduction) is used. Mathematically, what is recorded in such a situation is the second difference between the voltage of the central

electrode and the voltage at the two ends of the restricted extracellular space. If the restriction is cylindrical in nature and the electrode is placed at the geometrical center of the cylinder or tube, then the voltage recorded will be (Stein and Pearson, 1971, Eqn. 14)

$$V(t) = c\{\tfrac{1}{2}V_m(t + \Delta t) - V_m(t) + \tfrac{1}{2}V_m(t - \Delta t)\} \qquad \text{Eqn. 2.4}$$

where V_m represents the intracellular potential in a nerve fiber, $\Delta t = l/2v$, l is the length of nerve in the restricted extracellular space and v is the conduction velocity of the nerve fiber. The form of the intracellular potential and the constant c can be determined by classical monophasic recording methods.

Monophasic compound action potentials may be recorded from a severed nerve by placing one electrode near the cut end and locating the other appropriately on the intact portion of the nerve. Similarly, biphasic recordings can be recorded from cut or intact nerves by placing two functional electrodes on the normally conducting nerve. Although there are decided advantages of recording monophasic and biphasic potentials and these will be discussed later (Chapter 3), neither of these could match the performance of a balanced tripolar configuration in rejecting EMG. So for the present case of intact nerves, only the triphasic action potentials were recorded and monitored over varying periods of time.

The dependence of amplitude on the cuff length has been described elsewhere (Stein *et al.*, 1975, 1976; Hoffer, 1975) and will only be mentioned briefly here. Triphasic potentials increase in

amplitude as the square of the inter-electrode spacings and eventually approach a predicted value nearly 1.5 times the potential of a monophasic signal (Stein and Pearson, 1971). Generally, at cuff lengths of 3 cm or so, the amplitude saturates and increases in length beyond this value do not improve the signal levels markedly. The frequency spectrum of such signals is an important factor in choosing the appropriate cuff length. For example, the peak frequency of a neural signal will depend on the length of the cuff over which the potential is developed. A neural waveform, with peak-to-peak duration of t_p msec, may be expected to have a peak frequency component f_p (in KHz) of

$$f_p = \frac{1}{2t_p} \qquad \text{Eqn. 2.5.}$$

The waveform is relatively independent of cuff length over small values but begins to spread out markedly over longer lengths. This will in turn lead to lower frequency components of neural signals and hence render them more difficult to distinguish or separate from the EMG. It is clear, therefore, that in order to optimize the neural records, a number of different factors have to be considered and weighted appropriately.

The stability of the neural signals over time was studied by the methods already described whereby a stimulus was applied through one cuff and the evoked compound action potential recorded triphasically through another cuff. Fig. 11 shows the recordings, under anaesthesia, over a period of 6 months. Measurements were made from the sural nerve cuff while stimulating the sciatic nerve to produce a maximal compound

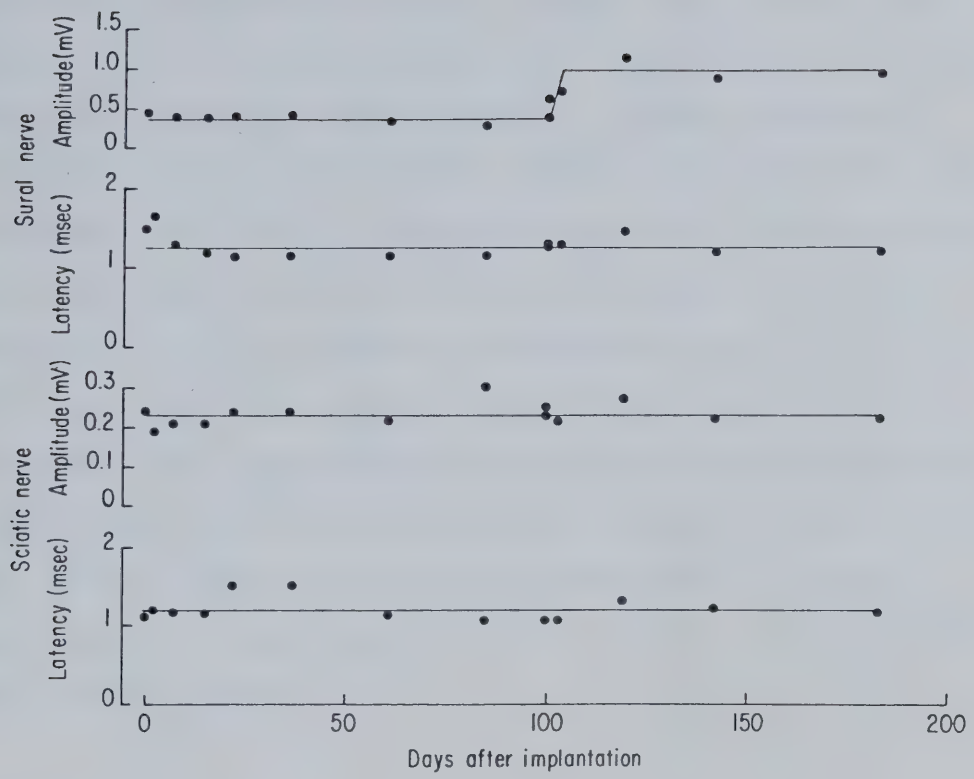


Fig. 11. Peak-to-peak amplitude and latency to first peak recorded in a cuff around the sural nerve (when stimulating the sciatic nerve) and in a cuff around the sciatic nerve (when stimulating the sural nerve) over a period of 6 months after implantation. The horizontal lines represent the mean values over the period of time indicated. No significant trends are observed. Due to the sural amplitude being smaller than expected, a second operation was performed at 100 days. Connective tissue had grown into the slit along the cuff. This tissue was removed and the cuff sealed more effectively. The amplitudes recorded more than doubled following this.

action potential. The sciatic nerve was then recorded from by back-stimulating through the sural nerve. Straight lines in Fig. 11 give the mean values over the periods indicated. Due to an unexpectedly low amplitude of its compound action potential, a second operation was performed to expose the sural nerve. The slit along the cuff was found to have been ineffectively sealed during the original implant and the connective tissue had invaded the cuff. After better sealing during the second operation the amplitude increased almost two-fold while no effects was observed on conduction velocity or amplitudes recorded at the sciatic cuff. Neural potentials up to several mV were recorded from longer lengths of nerve and these remained stable for varying periods of time. Table 1 summarizes the length of implantation for a number of cuffs together with the reasons for the termination of these experiments involving intact nerves.

(ii) Rejection of EMG

The basic principles underlying the rejection of EMG have already been outlined (see Introduction) and will now be discussed in light of experimental findings. When a potential is recorded at the center of a cuff with respect to an electrode immediately outside the cuff (Pin 12 or "ground" lead), two distinct potentials are recognized (Fig. 12A). An early neural peak is followed by a much larger, longer duration compound action potential. This is the EMG picked up from the muscle groups in the vicinity of the cuff and is characterized by its longer latency (due to time delays involved in neuromuscular transmission) and duration (from its greater membrane capacitance). If, however, two

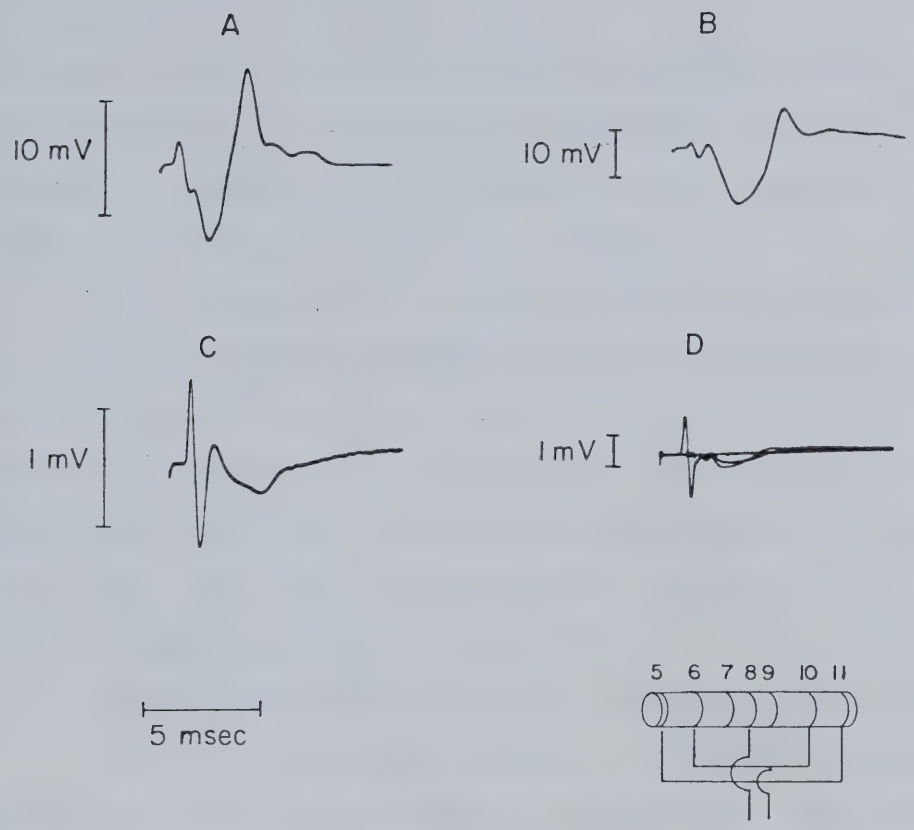


Fig. 12. Stimulating the sciatic nerve produces a compound action potential in the tibial cuff which contains fast, early neural potentials and slow, late muscle potentials (EMG). When recording A) between one electrode within the cuff, or B) between two electrodes within the cuff, the EMG dominates the recordings. When recording tripolarly C) between a central electrode and two end electrodes shorted together, the neural signal dominates. Better EMG rejection is obtained if further shunting is incorporated. D) shows the effect of recording from the center electrode (Pin 8) with two of the adjacent electrodes shorted (Pins 6 and 10). In addition, if the ends are shorted (Pins 5 and 11), the EMG is further reduced. Both traces are superimposed. The reasons for this are discussed further in the text. Recording bandwidth 10 Hz to 10 KHz.

electrodes on the intact nerve are recorded from differentially, the neural-to-EMG ratio is even worse (Fig. 12B). The failure of the common-mode rejection technique can be best explained by the fact that the EMG recorded at the two electrodes is not identical in form and is therefore not rejected by the differential amplifier, which relies on consistency in waveform of signals to be rejected.

The improvement with a tripolar configuration is enormous (Fig. 12C) for reasons already mentioned. The importance of shorting out EMG currents is further underlined in Fig. 12D, where further shunting is incorporated across the cuff ends. Pins 5 and 11, which are the end leads for a tibial nerve cuff, are shorted together, in addition to Pins 6 and 10 that are 1 cm into the cuff at each end. Pin 8 is the central electrode, being 2.5 cm away from each of the end leads, 5 and 11. A double shorting bar-type effect is provided by this arrangement.

Fig. 13 illustrates the time course of the EMG in several different cuffs under tripolar recording configurations. From these records it is evident that the EMG rejection worsens with time and in most cases appears to stabilize at some point. Some of this change in the pattern of compound EMG potentials can be attributed to the variability of leg position during recording and also to the position of the nerve cuff with respect to the different muscle groups. However, the rise in EMG is to a considerable degree correlated to the proliferation of connective tissue, which replaces the fluid in the cuff. Indeed, the nerve axons themselves and the tissue ingrowth could serve as effective channels for passively "piping in" the EMG.

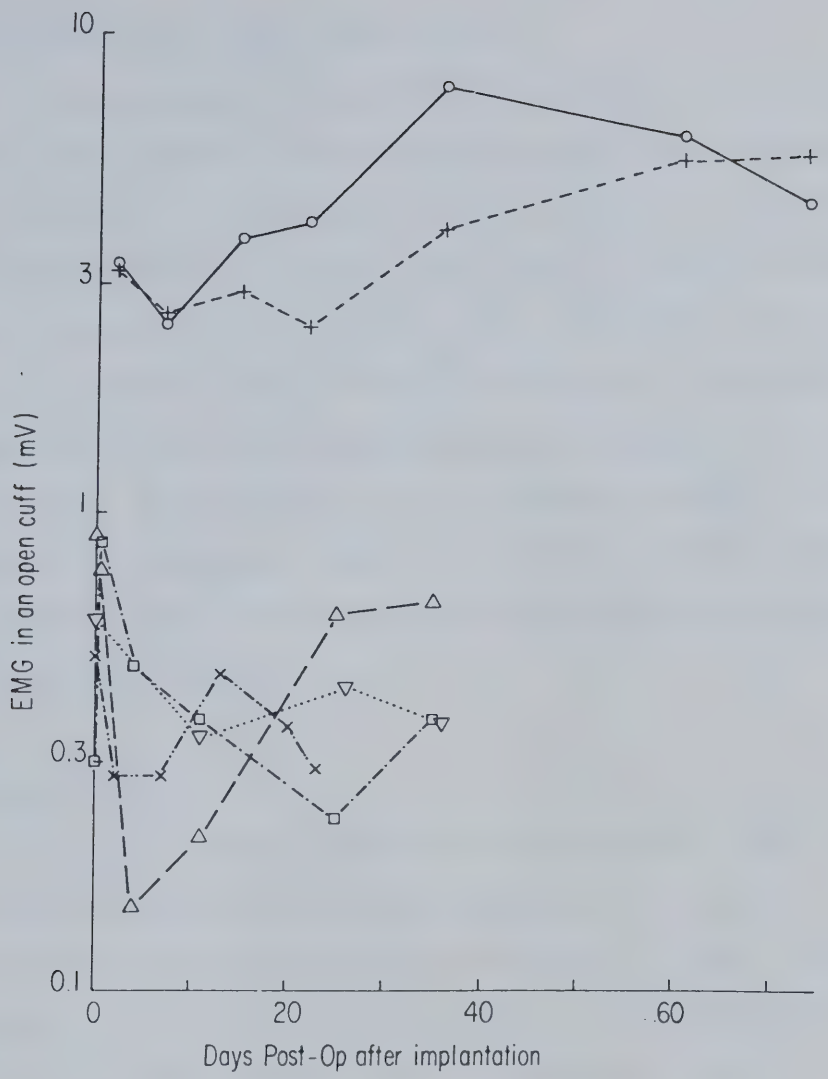


Fig. 13. Peak-to-peak amplitude of EMG recorded over time using tripolar configurations in cuffs around intact nerves. The nerves used were: X and ▽, tibial nerves; O, sural nerve; + and □, common peroneal nerves; △, LGS nerve. The increase in EMG over time corresponds closely with the proliferation of connective tissue into the cuff. Two cuffs (O and +) which exhibited unusually large values of EMG were found to be improperly sealed.

E. EVOKED SENSORY AND VOLUNTARY NERVE ACTIVITY

The data gathered during treadmill locomotion and by natural stimulation under anaesthesia was treated and analyzed in an identical manner. In addition to addressing ourselves to the central problem of partitioning neural activity, answers were sought to a number of specific questions related to the optimization procedures described earlier. For instance, the frequency spectrum of these signals was of considerable importance in helping to incorporate appropriate filtering into the recording set-up. Also of interest was the question of whether correlating neural activity within a single cuff (with two tripolar configurations) yielded any better results than performing the same operation between two individual cuffs.

(i) Analysis of Data

The spectral analysis package (French and Holden, 1971; French, 1973) was used to compute the input and output spectra, the cross-spectrum and also the coherence function for any pair of inputs and outputs (analog or digital). The prerecorded data was played back, at appropriate speeds, into a Digital PDP-8 Lab computer. Since the computer did not accept inputs exceeding ±1 volt, the two analog signals were often fed through a pair of operational amplifiers. Program AUTO was used to perform fast Fourier transforms upon the sampled data and also computed the spectral estimates from the Fourier coefficients. The spectra of the two neural channels and the cross-spectrum were printed and displayed in the frequency domain. To obtain the cross-correlation function as a time series, a computer program, SAP 3, was

used to carry out inverse Fourier transforms.

Fig. 14 shows a typical power spectrum of the two signals recorded from cuff electrodes on the sciatic and the common peroneal nerves of a cat while it was walking. The neural component of the signal, characterized by its higher frequency, is dominant on both channels, whereas the low-frequency EMG peak is identifiable on the sciatic trace only. From such records appropriate frequency cuts may be determined to eliminate the residual EMG and, in general, low-frequency cuts of 300-500 Hz were used in the filtering stages.

In cats under anaesthesia, it was possible to isolate and reliably identify the sensory component of the evoked sensory activity. Fig. 15A shows one such cross-correlogram with a sensory peak only. During simple behavioural tasks such as treadmill walking, cross-correlograms were obtained for the afferent and efferent contributions. These cross-correlograms (Fig. 15B) were distinct and segregated on opposite sides of the time record, allowing a simultaneous study of the motor and sensory components associated with walking activity. τ_s and τ_m represent the time delays for the sensory and motor waveforms, which were recorded from the two nerve cuffs.

If, however, the activity recorded from two configurations within the same cuff was cross-correlated, the noise level associated with the records was considerably reduced (Fig. 15C). Where possible, therefore, two tripolar configurations within the same cuff were recorded from, but the short lengths of most peripheral nerves in the distal hind-limb precluded this. In fact, the posterior tibial nerve was the only nerve used to date for this type of recording configuration.

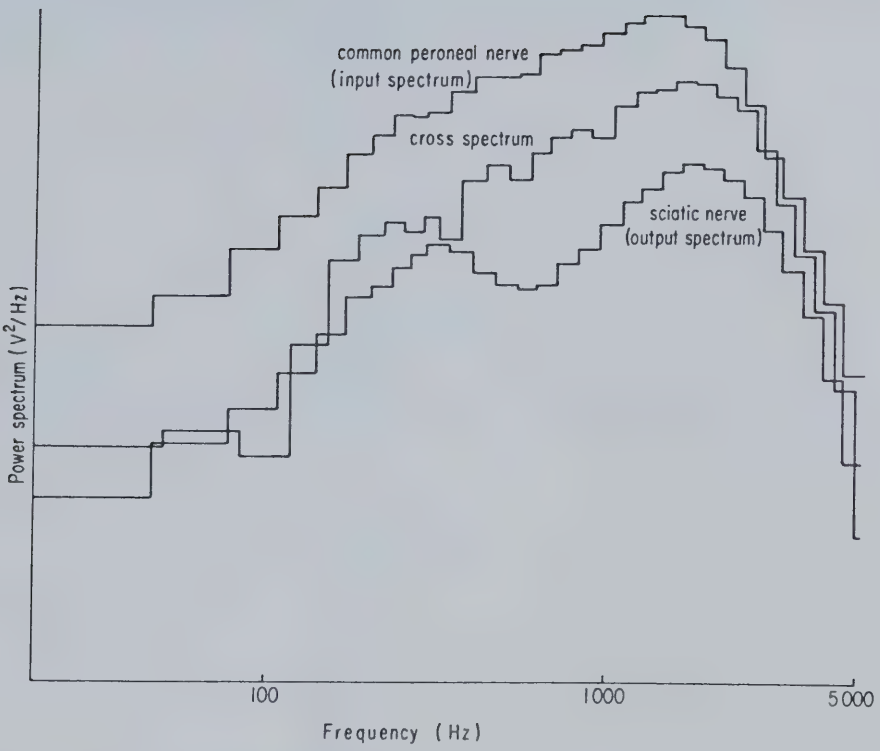


Fig. 14. Power spectra of signals recorded from two cuff electrodes while a cat was walking on the treadmill. The common peroneal signal (input) is almost purely neural but the one recorded from sciatic nerve (output) shows an EMG peak between 100 Hz and 1000 Hz. The cross-spectrum indicates the correlation between the two signals in the frequency domain. Units for spectrum are in V^2/Hz.

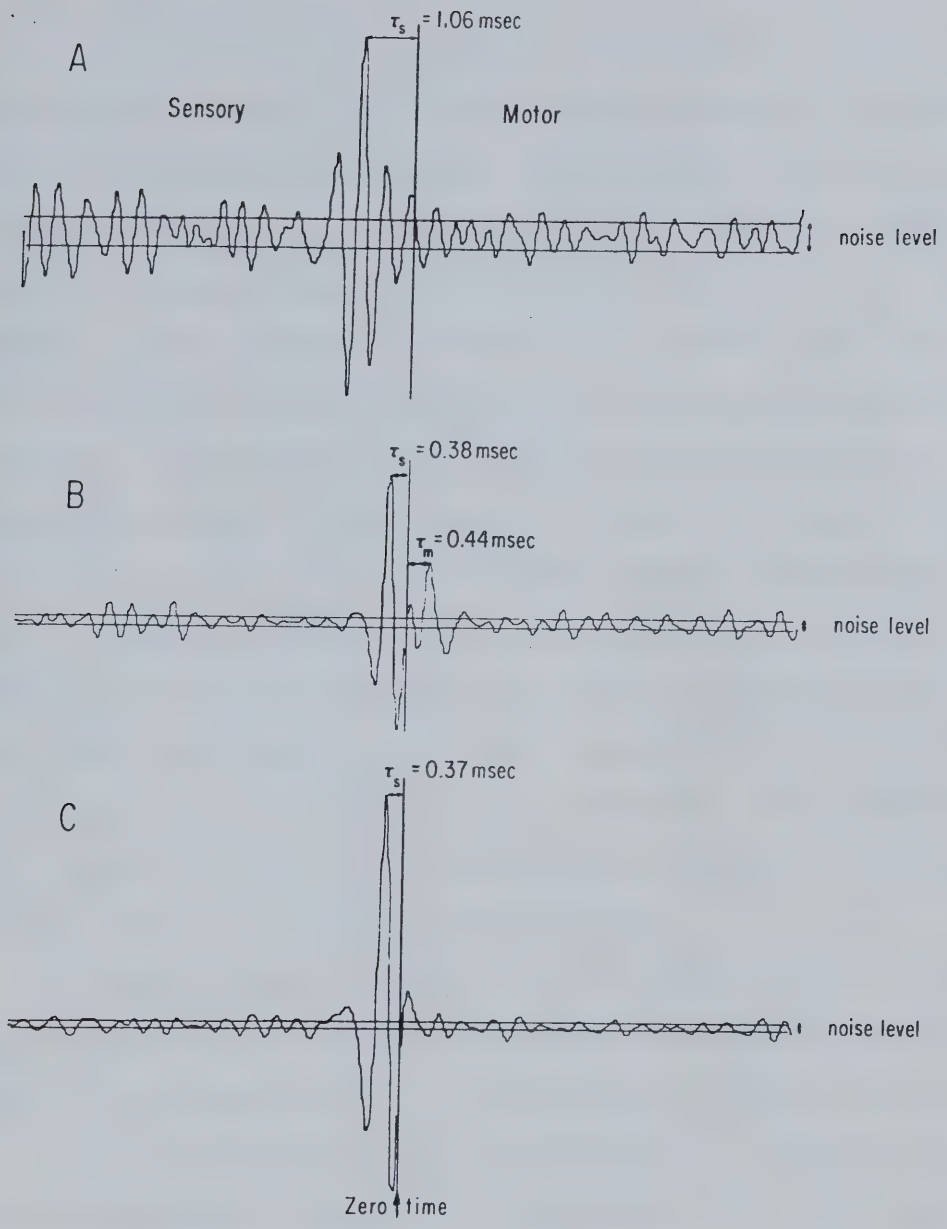


Fig. 15. Cross-correlation between signals recorded from two different nerves (A and B) or two different configurations on the same nerve (C). Neural activity recorded from a cat under anaesthesia shows only a sensory peak (A and C) whereas that recorded during treadmill walking shows both a motor and a sensory peak. The motor and sensory delays between the two recording contacts are indicated as τ_m and τ_s, respectively. Note the difference in noise level between A and C (for explanation see text). The nerves in A are sciatic and tibial; in B common peroneal and sciatic; and in C the tibial nerve.

In order to follow the dynamic response of each group of nerve fibers (motor or sensory), an on-line cross-correlator was constructed. The details of the integrated circuit incorporating the Analog Multiplier are shown in Appendix II. The function of this circuit was to simply multiply two analog signals, be they sine waves from a generator or two channels of neural activity. The insertion of a digital delay line on one of the inputs allows the multiplier to be used as a hardwire cross-correlator. The schematic in Fig. 16 illustrates the analysis for sensory activity with the delay line on the distal nerve channel. For motor activity, the digital delay is simply switched to the proximal cuff or electrode configuration. The correlated activity is appropriately amplified, filtered and displayed on a pen recorder (Hewlett-Packard, Model 7700). Simultaneously, the flexor or extensor EMG is filtered and displayed on a second channel of the pen recorder. Any modulation in the pattern or motor or sensory activity may therefore be correlated with the particular EMG bursts during locomotion.

The preliminary tests of the correlator were carried out on neural data obtained by natural stimulation of the hindlimb while the animal was under anaesthesia. The cross-correlated sensory nerve activity as a result of different stimuli is shown in Fig. 17. A range of delays, based on the computer cross-correlogram, was used. From the records, it is evident that different receptor groups are activated during the variety of natural stimuli applied to the tibial nerve and its peripheral field. This is not altogether surprising since the tibial nerve comprises of a mixture of muscle and cutaneous afferents as well as joint receptors. The difference in conduction velocity between these axonal

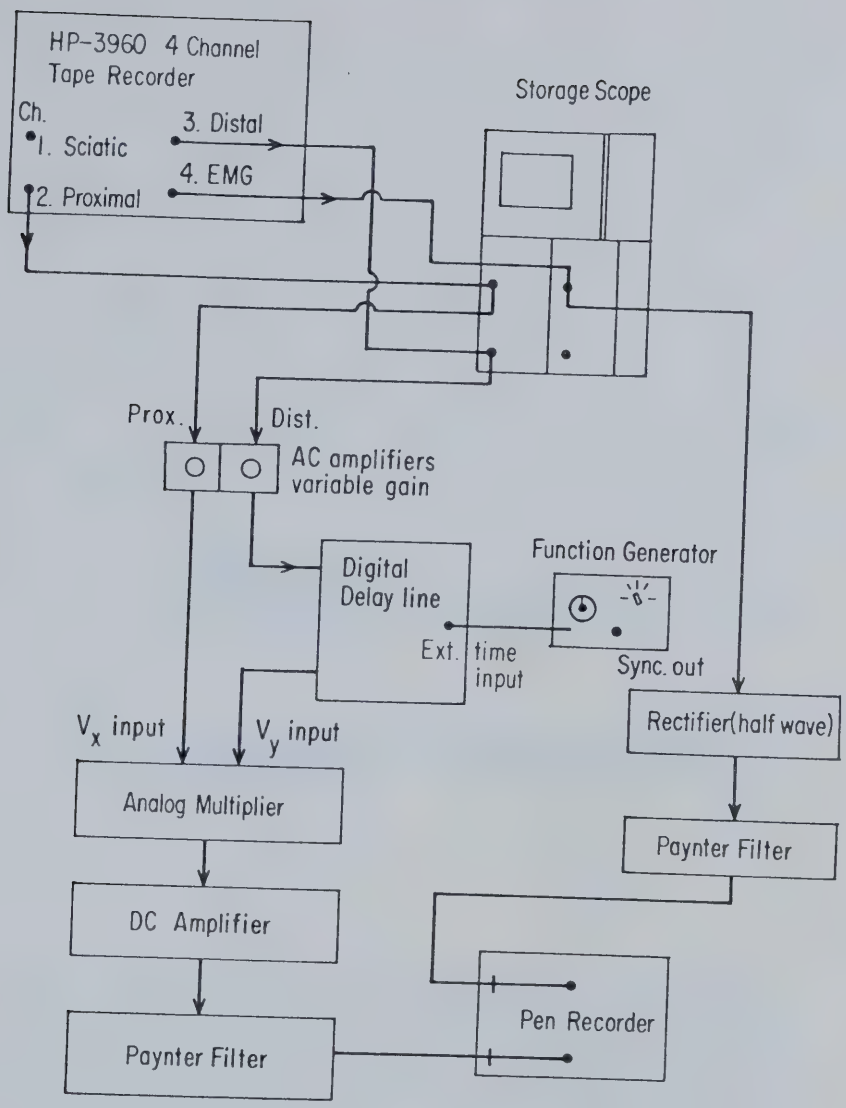


Fig. 16. Schematic for on-line cross-correlation of sensory activity. A range of time delays is available from the digital delay line (+5 V to =5 V). For cross-correlation of motor activity the delay line is inserted on the proximal channel instead of the distal one as indicated in this Fig.

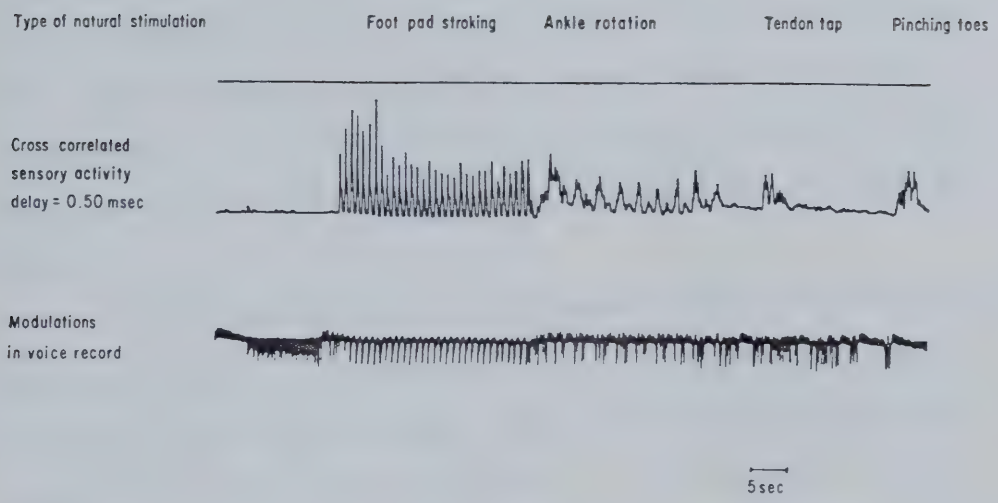


Fig. 17. Output of the on-line sensory cross-correlation scheme shown in Fig. 16. A delay of 0.5 msec is used on the data recorded from two nerves of an anaesthetized cat in response to a variety of natural stimuli. Afferents involved in the withdrawal reflex (pinching toes) and the stretch reflex (tapping tendon) are selectively activated. Different receptors (joint, cuteanous) are also activated in response to the appropriate stimuli (ankle rotation, skin stimulation). The Fig. shows the tape recorded data replayed at one-quarter of the original speed (15"/sec).

groups manifests itself in the cross-correlation record. Although the technique showed promising signs of segregating sensory activity during passive manipulation of the foot, less success was achieved in the analysis of the neural activity during locomotion. While Fig. 18 shows the capability of distinguishing between motor and sensory activity, no further differentiation within each of these groups is evident. A number of peripheral nerves, including some of purely muscle origin, were used in attempts to separate the sensory components. These endeavours met with little or no success and were subsequently abandoned. It is worth, however, contemplating the reason(s) for this lack of success.

The further partitioning of sensory activity by time convolution techniques requires that the axonal groups to be segregated possess different conduction velocities. This is not the case for several sensory fiber groups such as the Ia's, Ib's and β's, which all have overlapping ranges of conduction velocities. To resolve such differences in velocity requires a fairly long length of nerve over which to correlate the activity. In fact, our calculations show that in order to distinguish between afferent conduction velocities of 75 m/sec and 55 m/sec (based on data by Boyd and Davey, 1968) requires a separation of recording electrodes by 4.4 cm. Even longer lengths of nerve are required for fiber groups having a greater overlap of conduction velocities and few, if any, nerves can meet this criterion, at least in the cat hindlimb.

Attempts to distinguish the asynchronous motor activity, i.e., separate γ's from α-fibers, is a more difficult proposition in view of the fact that the γ's are well into the noise levels of our present

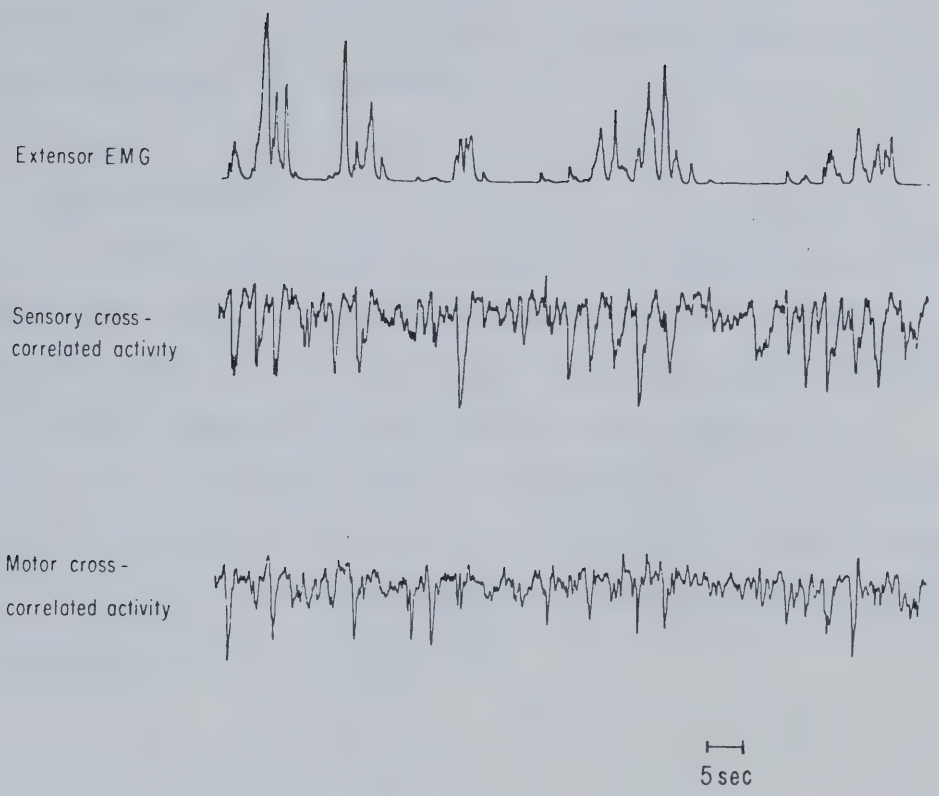


Fig. 18. On-line cross-correlation of motor and sensory activity recorded from the tibial nerve of a cat walking on a treadmill. The sensory peaks serve as reliable markers for foot contact with the ground. The close correlation of these peaks with the EMG activity during the stance phase supports the fact that the EMG recorded outside the cuff is mainly from the ankle extensors. The motor peaks provide a good method for measuring voluntary activity (see Chapter 3). No further partitioning of the motor and sensory components is obvious. 0.5 msec is the motor and sensory delay; data played back at reduced speed as in Fig. 17.

recording set-up. It may yet be possible to achieve some of the aforementioned aims, although perhaps in a different, more carefully controlled preparation. We have, however, managed to acquire some secondary benefits from the methodology of cross-correlation, and these are elaborated upon in Chapter 3.

F. A NOTE ON HISTOLOGY

As indicated in Table 1, several of the cuff electrodes were removed in order to make histological observations of the nerves. Although no gross abnormalities in these nerves were found, there was a preferential reduction in the numbers of large diameter fibers (Stein *et al.*, 1976; Hoffer, 1975). This phenomenon may have been the result of compression by the cuff and/or the connective tissue ingrowth. It is believed that the largest diameter fibers are the most susceptible to compression (Strain and Olson, 1975).

TABLE 1

Performance of Cuffs on Intact Peripheral Nerves (Nov. 1974 - Mar. 1976)

Cat #	Duration of Implant	Nerve cuffs (Pt-Ir wires)	Reasons for termination of experiment		
			Lead breakage	Pressure block	*Other
23	183 days	sciatic, sural and common peroneal			X
					X
		LGS	X		
32	82 days	sciatic			X
		tibial	X		
41	23 days	sciatic, tibial			X†
42	25 days	sciatic, LGS and common peroneal			X†
		(Ag wires only)			
22	nearly 8 months	sciatic, LGS, sural and common peroneal	X		
21	4 months	sciatic, common peroneal	X		
19	7 days	sciatic, sural		X	

* Other denotes experiments that were terminated for one or more of the following reasons:
a) histology required
b) functioning cuffs and nerves when removed but no further physiological data required
†c) the nerves were cut and capped to study the response to axotomy

CHAPTER 3

ON THE FATE OF NEURAL ACTIVITY IN SEVERED PERIPHERAL NERVES

The extension of the methods described in the previous chapter to the study of cut nerves served a dual purpose. Firstly, for any prosthetic applications, it was important to ascertain the feasibility of recording neural activity from severed nerves for prolonged periods of time, as would be the case in an amputee. The second and perhaps a more basic purpose was to monitor electrophysiologically any changes in the state of peripheral nerves following axotomy.

Although the subject of peripheral degeneration and regeneration has been thoroughly and frequently reviewed between 1892 and 1975 (Ranson, 1906; Guth, 1956; Cragg, 1970; Grafstein, 1975), the fate of severed nerves is still controversial. It is, however, well known that when an axon is cut, the nerve cell body undergoes certain profound alterations in structure, metabolism and physiological activity. The typical morphological changes include swelling of the cell body, migration of the nucleus from the axon hillock to the cell margin and the disappearance of stainable Nissl granules from the cytoplasm. The temporal sequency of these so-called chromatolytic changes is paralleled by an exhaustion of the nucleotides and proteins within the cell body. Some investigators (Hydén, 1943; Cragg, 1970) have attempted to explain these biochemical alterations in terms of increased metabolism by the cell body to offset axoplasmic loss from the cut end and to initiate efforts at regeneration by the axon.

While these points have been substantiated and well documented

in texts and review articles (Cajal, 1928; Guth, 1956; Jacobson, 1970), it is worth reviewing the labyrinth of conflicting evidence on the functional capability of the nerve following axotomy. As early as 1892, Marinesco, in a pathological examination of an amputee's spinal cord, remarked on the atrophic condition of the dorsal root ganglion cells. He also commented on the fact that the pathology and depletion of the cells (to about a third of the values on the control side) in the lumbo-sacral region of the cord was by no means restricted to the dorso-lateral group, but also extended to the group of cells in the ventral horn of the afflicted side. Subsequent observations at the turn of the century seemed to confirm the view as expressed by Warrington (1899): "I do not doubt that the reaction of a cell to a lesion of its axon is in part due to the abolition of certain afferent impulses; when however these are *all* cut off, we find individual cells are more severaly affected and on following their ultimate fate many disappear." The retrograde degeneration resulting from the transection of the spinal nerves was extended to include the cells of Clarke's column and the dorsal column (Warrington, 1899). Ranson (1906), in an extensive review of earlier work, points out the differences in the atrophic nature of the different cell groups. However, all fibers experiencing the degenerative changes were reduced in diameter and many lost their myelin sheaths. In his own experiments, he carefully enumerated the spinal ganglion cells remaining as a result of cutting the second cervical nerve of the rat. His findings revealed that a constant number (about one-half) of the cells in the corresponding ganglion degenerated and disappeared, whereas the loss of cells in the ventral horn was quite variable.

The later studies differ in their estimates of the number of cells perishing after nerve division. Cavanaugh (1951) reported a loss of 50% in the spinal ganglia and the losses for cells in the anterior horn vary from 75% (Watson, 1965) to 6% (Barr and Hamilton, 1948). Cragg and Thomas (1961), in one of the very few electrophysiological studies on the subject, showed that when the distal end of a nerve is avulsed, the conduction velocity proximal to the site of division falls to 60-70% of the normal within 200-400 days. Concomitant with this is a reduction in total fiber diameter. This study seems to suggest that the central stump of an avulsed nerve is still capable of impulse propagation a year or so later, albeit with a reduced conduction velocity.

In spite of the conflicting evidence on the extent of nerve cell death following axotomy, certain salient features derived from these earlier studies are of significance.

1) The rate and the extent of chromatolytic changes vary greatly in different neuronal groups and also within the same functional group.

2) The intensity of the chromatolytic reaction varies inversely with the distance of the site of lesion from the parent cell body.

3) Sensory neurons are affected more rapidly and severely than their motor counterparts.

4) The recovery of the neuron from chromatolysis depends upon the severity of the injury and also upon the reunion of the central and distal stumps of the severed nerve. The later point appears to be contentious amongst the various investigators.

The question that remains unanswered is, can severed nerves continue to generate action potentials in the absence of functional connectivity to an end organ? Clinical experience indicates that afferent fibers remain viable for prolonged periods of time and can produce painful neuromas. Also known is the fact that severed nerves can be surgically resutured to restore some motor function and that this type of an operation is feasible for a year or more after the injury. Recently, DeLuca and Gilmore (1976) claim to have recorded voluntary neural signals from severed nerves of rabbits for over 3 weeks. In addition, they were able to record evoked compound action potentials from such nerves for some months. However, no indication is given of the changes in amplitude of these signals over time, nor is there a reference to any discrepancies that may exist between the patterns of voluntary and evoked nerve activity.

Using the optimal recording methods described previously, we have attempted to shed some light on these hitherto unresolved questions. Severed peripheral nerves were placed in a cuff whose distal end had been sealed. Sequential changes in the state of the nerve fibers were monitored by recording impedance of the nerve, the compound action potentials and voluntary activity at various distances from the sealed end of the cuff. Although our primary aim in sealing the distal end of the cuff was to improve the EMG rejection, the idea of encasing severed nerve ends in rigid tubes is by no means new. Several successful attempts at preventing neuroma formation using the encasement method have been reported (Poth and Fernandez, 1944; Edds, 1945), but the clinical value of such a procedure is dubious (L. Davis, personal communication). We also studied the

effects of various cuff sizes on the amplitude of neural signals in order to determine the optimum ratio between cuff size and the nerve diameter. A tight fitting cuff improves the signal size markedly and this is particularly important for recording small amplitude neural signals. However, allowance has to be made for swelling of the nerve following axotomy and the occurrence of a pressure block is not uncommon in overtly tight fitting cuffs.

The recording methods used for measuring the various parameters were basically the same as those in Chapter 2 but with some exceptions, particularly in the treatment of the data.

A. RECORDING METHODS AND ANALYSIS OF DATA

(i) Impedances

Since the measurement of impedances proved to be of considerable use in monitoring the state of the intact axon over time, the practice of measuring them regularly was continued for severed nerves as well. Of particular importance was the impedance of each electrode measured with respect to an indifferent or a ground electrode on the outside of the cuff. The difference in impedance between adjacent electrodes which were spaced varying distances apart (0.6 to 1.2 cm) gave the impedance of the tissue and fluid filling that space. As well, the impedances of various configurations used behaviourally were measured at various times after implantation.

(ii) Evoked Compound Action Potentials

If a nerve is tied, cut and placed in a sealed cuff, it is

possible to record a classical monophasic compound action potential when the nerve is stimulated more proximally (Stein and Pearson, 1971). This was done by using electrodes in one cuff for stimulation and recording differentially between electrodes in a second cuff. One electrode was located near the cut sealed end and the others were located at varying distances away from the ligature. Monophasic potentials were recorded regularly and their amplitudes plotted both as a function of time and of distance from the sealed end.

The compound action potentials recorded monopolarly showed a variable number of peaks in their waveform, which became quite contaminated with EMG over time. The latency measurements from such records were therefore quite unreliable. Consequently, biphasic potentials were recorded at varying distances from the cut end by placement of two electrodes on the nerve. The biphasic compound action potentials so recorded can be up to twice as large as their monopolar counterparts and merely reflect the difference between two recording sites, each of which registers a full monophasic action potential (Stein and Pearson, 1971).

Tripolar recordings were also made of the neural compound action potentials and the associated EMG in order to determine the best configuration for use in behavioural studies. The dependence of amplitude on inter-electrode spacing was also of interest and therefore plotted.

Although no rectal probe was used to monitor the body temperature of the anaesthetized animals, we did employ a controlled heating pad to keep the animals warm within a constant temperature range (37°-39°C).

(iii) Voluntary Activity

In order to quantitatively evaluate the voluntary activity before and after the nerve was cut, the cats were trained to perform a stereotyped behavioural task. From our experience on intact nerves, treadmill walking proved to be quite a suitable and reproducible index of voluntary activity. The cats were trained and recorded from in much the same manner as described in Chapter 2 (see Voluntary Activity; also Fig. 5). The recorded neural activity from the severed nerve was amplified, full-wave rectified, filtered and displayed on a pen recorder. Alternately, the voluntary activity from two channels was cross-correlated using a range of motor delays. In either case, a modulation of neural signals with EMG activity (on a parallel channel) was displayed. The amplitude of the cross-correlated motor activity during steady stepping was measured on several occasions before and after cutting the nerve. With appropriate gains taken into account, an average value of the neural signals during ten or more steps was calculated. The voluntary activity for each occasion was normalized with respect to that obtained immediately prior to the nerve being cut. The index of voluntary activity so obtained was then plotted as a function of time.

B. CHANGES IN THE ELECTRICAL ACTIVITY OF THE NERVES FOLLOWING AXOTOMY

(i) Impedances

Shown in Fig. 19 are the variations in impedance for electrode spacings of 1, 2, 3, 4 and 5 cm from the sealed end of the cuff. All the impedance measurements were made at 1 KHz and therefore reflect changes

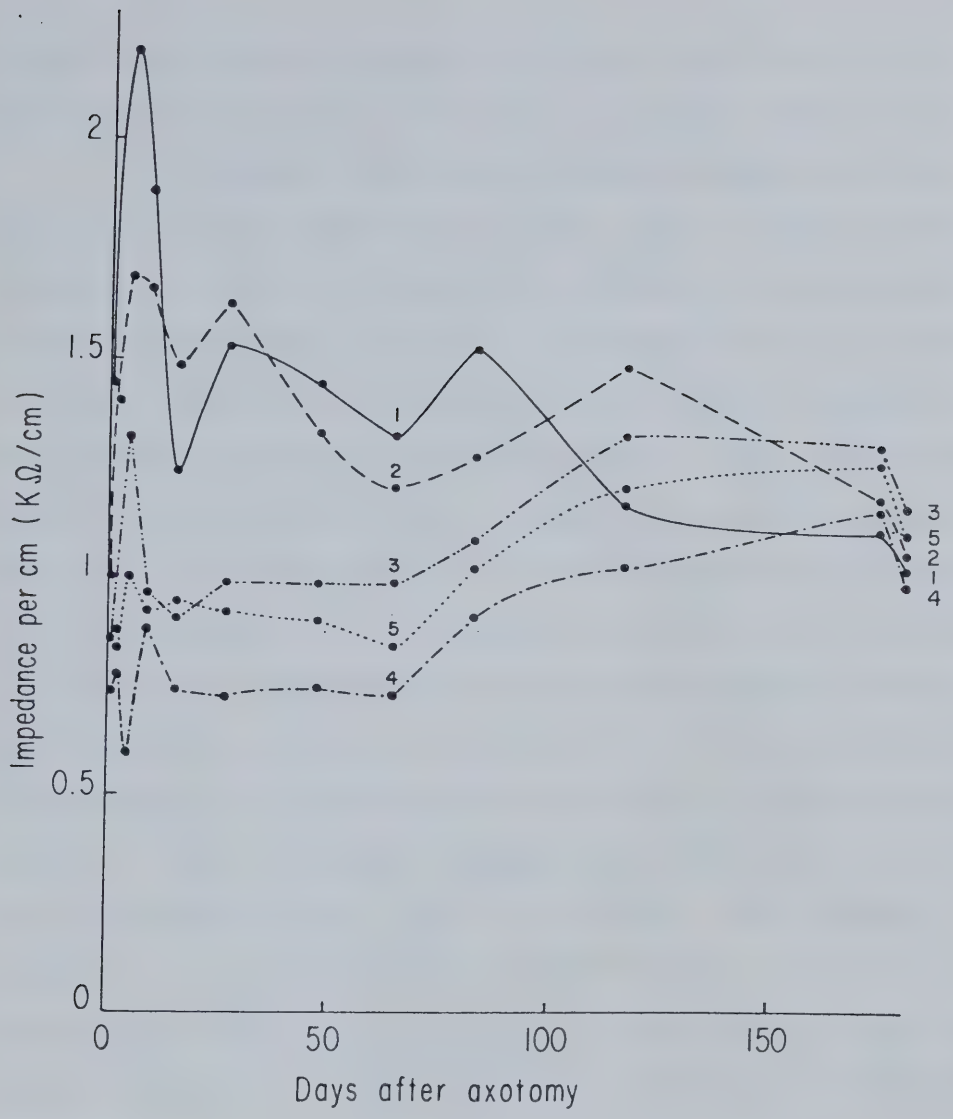


Fig. 19. Impedance changes over time at varying distances from the sealed end of a tibial nerve cuff. 1, 2, 3, 4 and 5 denote the distance in cms of electrode contacts from the severed end of the nerve. Initial changes are most pronounced closest to the sealed end. However, the later rise in impedances, due to connective tissue ingrowth, is more evident towards the open end of the cuff. Further details of the degenerative and regenerative processes underlying the changes are discussed in the text.

in the state of neuronal tissue within the cuff rather than in any electrode properties per se (see Chapter 2, Impedance Measurements).

The impedance in the first cm from the ligature increased sharply and reached a peak within less than a week. Further from the ligature the increase was slower and at a distance of 5 cm comparable to that of intact nerves. The more rapid increase close to the ligature is presumably due to the swelling of the nerve and can be explained by the blockage of axoplasmic transport from the cell body. Following this initial rise, the impedance shows a steady decline over the course of a week or so. This may be due to the axonal degeneration back from the cut end. A subsequent rise in impedance, though not quite as prominent as the first one, is observed to take place for a period of 7-10 days. This increase was greatest at a distance of 1-2 cm from the cut end of the nerve and could represent attempts by fibers to regenerate through the seal. Further increases in impedance were interpreted as being the result of connective tissue growth into the cuff. These were most prominent towards the open end of the cuff. However, the now well advanced degeneration, particularly at the distal end of the nerve, may also be responsible for some of the decreases observed in the impedance measurements.

(ii) Evoked Neural Activity

Unlike in the intact nerve, the compound action potentials recorded from a cut nerve placed in a sealed cuff do change with time. Marked changes are observed in both the amplitude and latency of the potentials and these are shown in Figs. 20, 21 and 22. The parallel

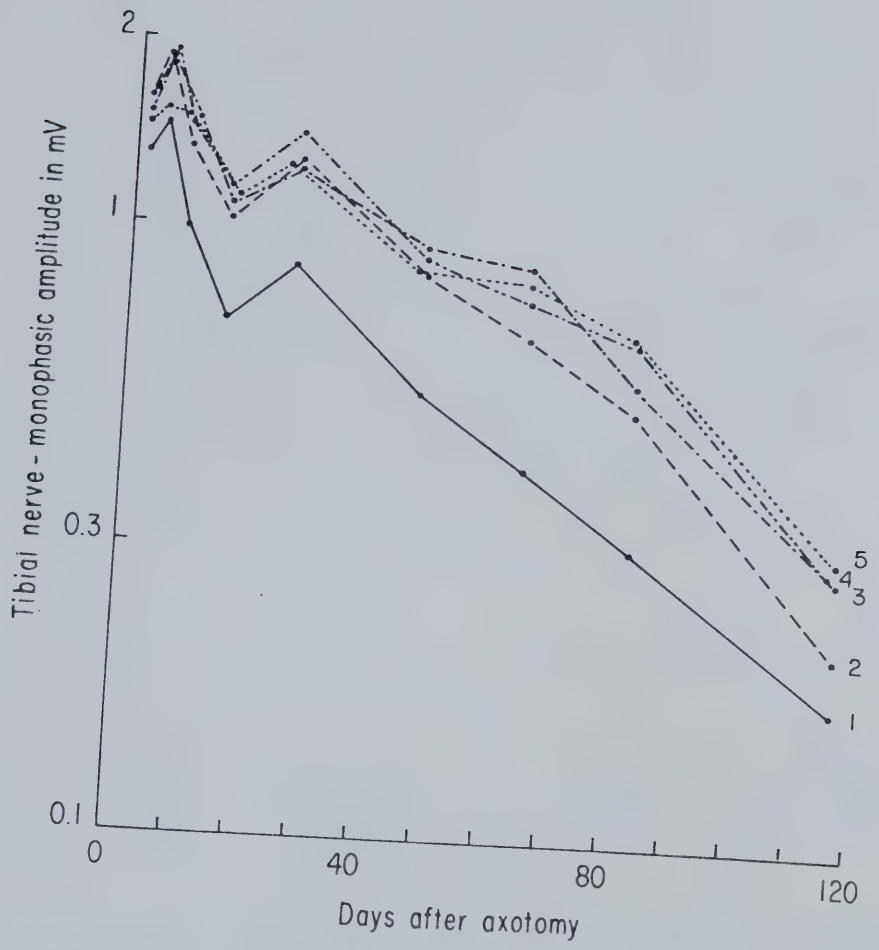


Fig. 20. Changes in the monophasic amplitude of the compound action potentials recorded at varying distances (1, 2, 3, 4 and 5 cms) from the severed end of the nerve. The sciatic nerve was stimulated and the recordings made from the tibial nerve cuff. Most dramatic changes take place closest to the site of axotomy (1-2 cms). The neural signal declined slowly with a time constant of 1-2 months. See text for interpretation of the initial changes.

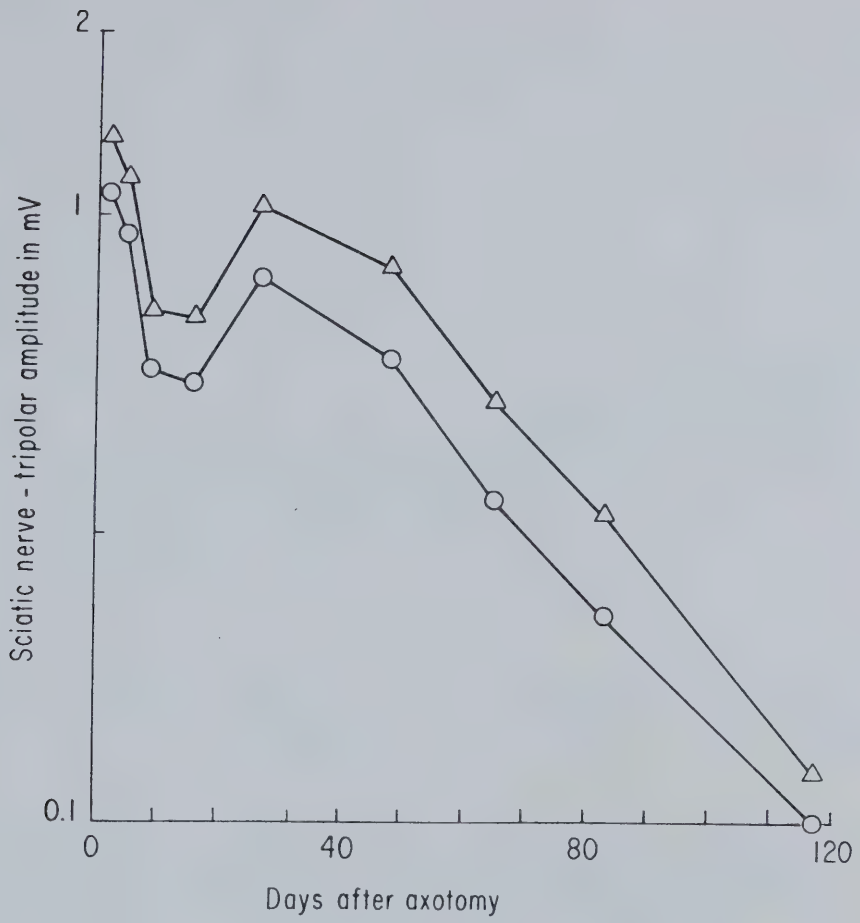


Fig. 21. Peak-to-peak amplitudes of compound action potentials recorded tripolarly from the sciatic nerve when stimulating the tibial nerve. O and Δ represent the sites of stimulation 1 and 4 cms respectively from the sealed end of the tibial cuff. The decline in amplitude is the result of the thinning of axons along the entire length of the nerve.

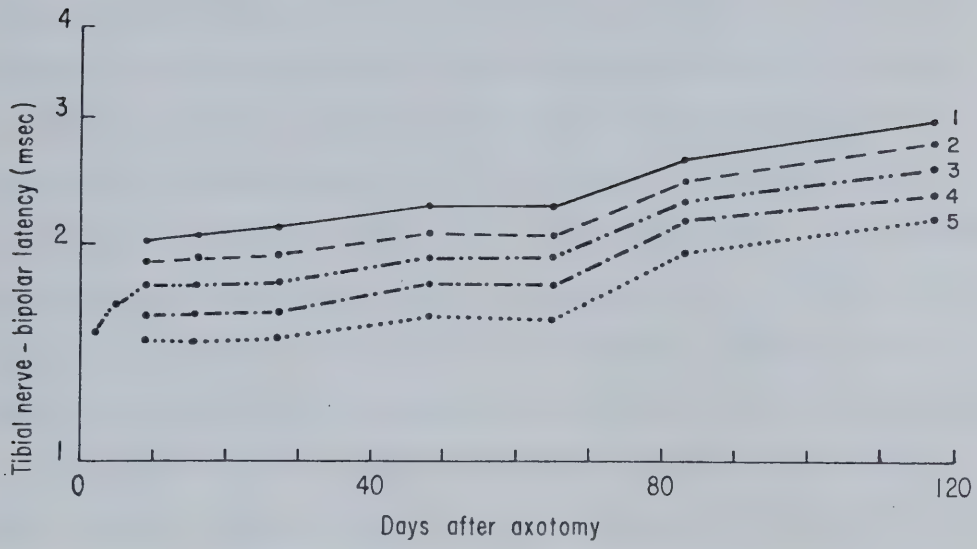


Fig. 22. Changes in latency to the first peak of bipolarly recorded compound action potentials at various distances from the sealed end of the cuff (as in Fig. 20). The thinning of axons and slowing of conduction account for the steady decline in latencies over time that is observed at all five points along the tibial nerve.

between the time-course of these parameters and impedances (Fig. 19) is striking.

As is shown in Fig. 20, the most dramatic changes take place close to the cut end. If the sciatic nerve is stimulated, the action potentials recorded from the cut tibial nerve grow sharply as the cut end swells. Then the potentials fall due to the degeneration which is taking place. Three cm away from the cut end the increase is less marked, but the effects of degeneration are clearly seen. At 5 cm from the sealed end, virtually no change is evident as yet. All the recordings shown were monophasic with respect to the cut end. However, if the stimulating and recording electrodes are switched, a triphasic potential can be recorded from the sciatic nerve while stimulating the tibial nerve. In this case not much of a change in potential is seen over the first few days, but the later decline is quite clear (Fig. 21). This is due to the fact that in addition to the degenerative changes close to the end of the nerve, the diameter of the fibers decreases along its entire length. The decrease in amplitude and an increase in latency measurements did in fact reflect this change (Figs. 20, 21 and 22). The regenerative phase of the fibers manifested itself by an increase in amplitude of potentials 2-3 weeks after axotomy. After this period the compound action potentials declined slowly with a time constant of 1-2 months. The conduction velocity also declined steadily, but more slowly. These findings were interpreted to represent a slow, continuous decline in fiber diameter of axons which could not find an end organ. This decline was observed to be more rapid in tight fitting cuffs, perhaps due to a selective effect of compression on the largest fibers (Strain

and Olson, 1975).

Two other phenomena associated with latency and form of the compound action potentials were evident on a regular basis. Over the first post-operative day the latency actually decreased (not shown in Fig. 22) as the animal recovered from the Nembutal anaesthesia. The cats did not regulate body temperature well while anaesthetized and recovery was marked by considerable shivering in order to regain normal body temperature. The effect of the progressive slowing in conduction and thinning of axons was to alter the shape of the potentials recorded over time. The compound action potential became more dispersed in time and developed multiple peaks that were initially not evident.

The dependence of the peak-to-peak amplitude of the compound action potentials on spacing between electrodes is shown in Fig. 23 for monophasic, biphasic and triphasic recording configurations. The findings are consistent with those reported earlier (Stein *et al.*, 1975, 1976) that the amplitude of monophasic potentials are quite independent of inter-electrode spacing unless the distance of the second electrode from the cut end is small, the reason for the reduction at short distances being due to impaired conduction close to the cut end of the nerve. Also shown in Fig. 23 is the linear increase in biphasic amplitudes as a function of electrode spacing. The triphasic amplitudes exhibit a steeper dependence on inter-electrode spacing and this underlines the usefulness of recording tripolarly over longer lengths of nerve, particularly as signals decline with time.

The problem of EMG rejection in a sealed cuff is of a somewhat different nature, since EMG currents do not flow through the cuff. Fig. 24

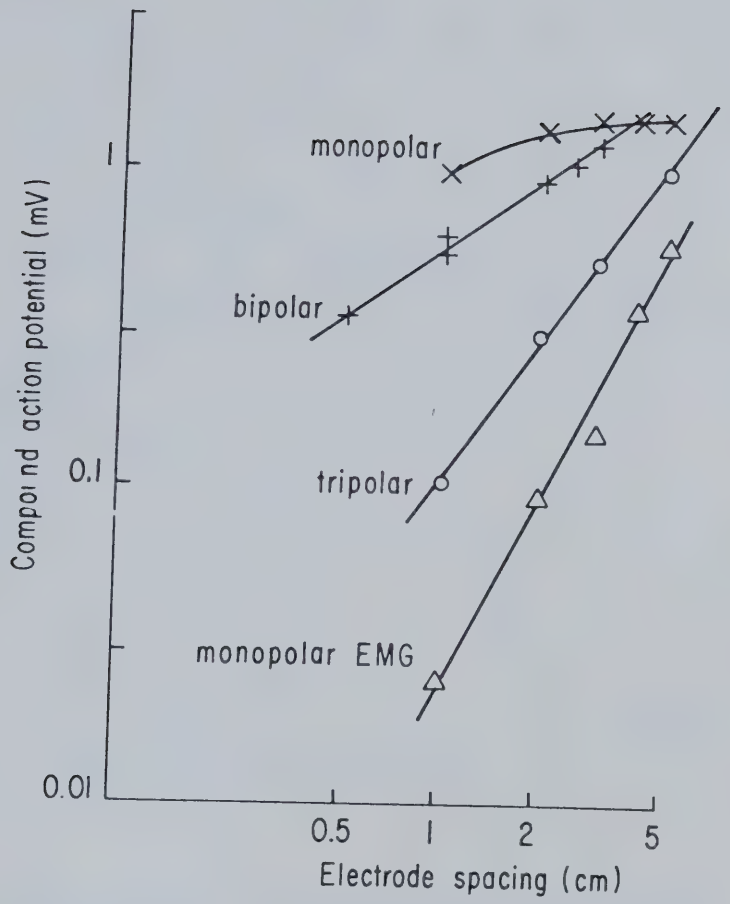


Fig. 23. Dependence of the peak-to-peak amplitude of the compound action potentials on the spacing between the electrodes for monophasic, biphasic and triphasic neural recordings and for monophasic EMG recordings. For monopolar records the x-axis also denotes the distance from the sealed end of the cuff. Both scales are logarithmic so that a line with slope of 2 implies that the monopolarly recorded EMG increases as the square of the distance from the sealed end.

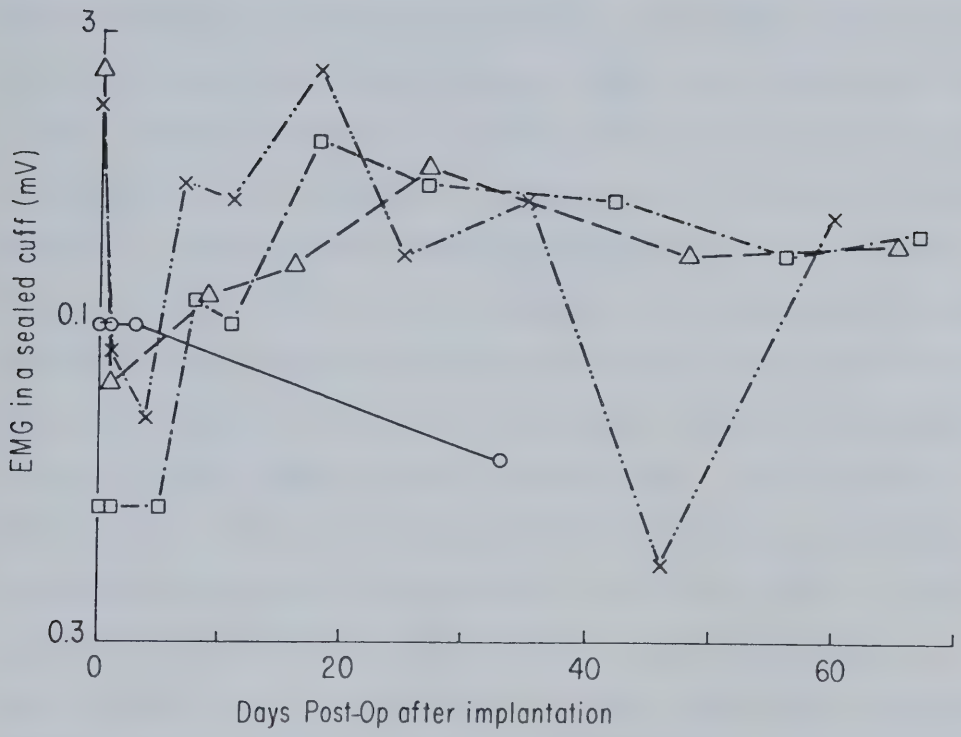


Fig. 24. Peak-to-peak amplitude of EMG recorded over time using tripolar configurations in sealed cuffs around cut nerves. The nerves used were: X and Δ, tibial nerves; □ and O, common peroneal nerves. The EMG amplitudes recorded from sealed cuffs are considerably lower than those recorded from open cuffs (see Fig. 13).

shows the behaviour of EMG recorded tripolarly from different nerves over time. If a similar plot for intact nerves (Fig. 13) is used for comparison, the immense improvement in EMG rejection with sealed cuffs is apparent. The absolute value of EMG recorded in cut nerves under optimum configurations is reduced by at least a factor of two, and in some cases as much as ten. The EMG potentials in a sealed cuff are caused by the transverse resistivity of the tissue and the voltage gradients set up in the radial direction. These potentials decrease rapidly with distance into the cuff, as is shown in Fig. 23. As opposed to the monophasically recorded neural amplitudes, which are fairly constant until near the cut end, the EMG signals appear to decline according to the square of the distance from the open end (slope of 2 on a log-log scale). The connective tissue growth into the cuff increases the resistivity of the medium, including the transverse component, and a rise in EMG levels is consequently observed (Fig. 24). After a month or so the EMG appears to attain stable levels, as in the case of intact nerves, and the relationship of EMG to distance shown in Fig. 23 is also unchanged, at least in form.

(iii) Voluntary Activity

It was our observation that voluntary activity did not continue beyond a period of about a month, even though the severed nerve was capable of being electrically stimulated to conduct action potentials for up to 6 months. The rapid decline of voluntary signals followed a pattern that was quite similar to the one seen for impedances and compound action potentials. In Fig. 25 are shown the changes in neural activity recorded from the tibial nerve before and after axotomy.

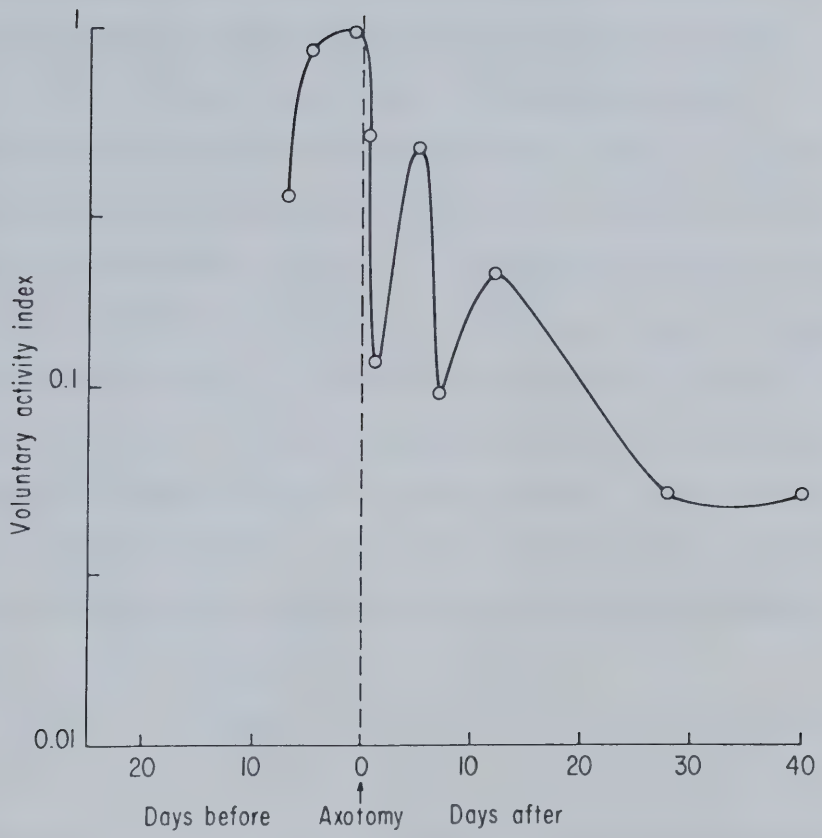


Fig. 25. Voluntary activity as a function of time before and after axotomy. The voluntary signals from a walking cat are normalized with respect to those recorded immediately prior to the operation. The changes are discussed in the text but note the over 25-fold drop in the neural activity within 28 days of axotomy. The evoked signals by contrast are still comparable to their values shortly after the operation. The significance of these results is elaborated on in the text.

The motor activity from the intact nerve showed a steady increase during three successive sessions of treadmill walking. This reflected the progress by a naive cat in performing the stereotyped walking task. The voluntary signals recorded post-operatively showed an initial decline, followed by two distinct phases of increased activity. The time course of these changes corresponded closely with those observed for impedances and evoked activity in the same cat. However, with 28 days an over 25-fold drop in the level of voluntary activity was observed, thereby making it indiscernible with our recording methods. In contrast to this, the evoked signals in the same animal were still large 28 days after the nerve was cut. From the foregoing results, it would appear that a cessation of voluntary activity may represent a morphological alteration of synaptic input to the axotomized motoneurons. In fact, the pattern of retrograde changes and its intensity at the segmental level may be a direct consequence of denervation.

As will be elaborated on in the next chapter, it was also our desire to elicit and record reflex activity from the severed nerves. Upon stimulation of the cut nerve, it was possible to record both the monosynaptic and the polysynaptic components of the reflex action from different muscle groups. Such reflexes were very much in evidence for periods of time up to 4 months and this would tend to suggest that afferent input to other motoneuronal pools was still intact.

It is appropriate now to discuss the significance of the present findings and to focus some attention on the current preoccupations of investigators in this area.

C. IMPLICATIONS AND DISCUSSION OF THE RESPONSE TO AXOTOMY

Over the years there have been many descriptions of the changes evoked by axotomy. As was pointed out earlier in this chapter, it is often difficult to draw sufficient information from these early studies since the criteria for chromatolytic reaction varied quite considerably amongst investigators. Also, much of the previous histological work has been shown to be inadequate for distinguishing the kinds of cells affected during axonal reaction or for diagnosing the nature of changes that these cells are undergoing. Some important questions have thus been left unanswered, for example, whether every type of neuron (sensory, motor, interneuron, etc.) exhibits the same response to injury. In lieu of this, any evidence on the basic sequence of events for different axotomized neurons must be welcomed. The virtual absence of any electrophysiological correlates of morphological observations on cut nerves emphasizes the need for an alternate index to study the responses to axotomy.

In this series of experiments we have been able to follow electrically the various stages in retrograde degeneration which have been well documented histologically. While the response to axotomy is quite variable, certain general features are consistently seen: the initial swelling close to the cut end due to accumulation of axoplasmic material (Jacobson, 1970), followed by a degeneration back from the cut end, an attempt by the fibers to regenerate and a final degenerative process if axonal sprouting is inhibited either with drugs or by capping the severed end. In any case, the retrograde degeneration of the nerve enclosed in a sealed cuff is complete by about 6 months. What is more

significant is the loss of voluntary signals within 4 weeks. This is in some contradiction to the findings of DeLuca and Gilmore (1976), who reported that voluntary activity persisted in severed peripheral nerves for up to 3 weeks. They also claimed that the amplitude of such activity stabilized after 3 weeks, although their recording methods did not permit them to extend their recordings beyond this point.

Recently a number of reports have shown that a considerable number of afferent terminals are sheared off from the cell body as a direct consequence of swelling shown to occur during chromatolysis (Mendell *et al.*, 1974; 1976; Purves, 1975). In the superior cervical ganglion of the guinea pig, Purves (1975) observed the loss of synapses following axotomy so great that many of the neurons were incapable of generating potentials even with maximal preganglionic stimulation. In a parallel study, Mendell *et al.* (1976) have shown that the failure of Ia connectivity to axotomized motoneurons took place within a period of 40-60 days and that changes in EPSP characteristics preceded the loss of synaptic boutons. Cull (1974, 1975), in a couple of anatomical studies, has confirmed the shedding of synaptic boutons from the surface of axotomized motor neurons in the hypoglossal nerve of the rat. Furthermore, his work suggests that the loss of afferent synapses is reversible if effective nerve-muscle contact is restored. An earlier study on the pattern of phrenic nerve activity following axotomy (Acheson *et al.*, 1942) would seem to confirm this view.

Taken together, these experiments suggest that the changes in reflex and voluntary activity of the nerves following axon inter-

ruption have a largely morphological basis in the form of synaptic disjunction. The results of the present study offer further support for this view. We find that the descending input from the higher centers, in the form of voluntary activity, closely parallels the retrograde degeneration in the peripheral nerves. The drastic fall in the level of voluntary signals would suggest a loss of afferent synapses from the axotomized motor neurons rather than some degenerative process manifesting itself at the presynaptic level. This hypothesis is supported by the ability of the severed afferents to sustain reflex action through normal motoneuronal pools for periods up to 4 months. The functional intactness of some presynaptic terminals is therefore beyond doubt.

We are, however, left with the unescapable fact that re-establishment of peripheral connections is of paramount importance in restoring synaptic integrity. Acheson *et al.* (1942) did indeed observe a decline in discharge of phrenic nerve activity for 21 days, but the subsequent restoration of activity was undoubtedly due to functional contact of the nerve with a peripheral target. It would therefore seem that neuronal death is inevitable in the absence of trophic interaction with an end organ and that the integrity of post-synaptic structures is dependent upon the trophic factors provided transneuronally, perhaps by the opposing presynaptic terminals. The implications of these results as they pertain to the neural control of artificial limbs will be discussed in Chapter 5.

D. HISTOLOGICAL NOTE

Histology was performed on the LGS, the common peroneal and the tibial nerves of cats in which neural activity had ceased. Sections of the nerve within the cuff showed almost complete degeneration with axons being sparse, the myelin deformed and evidence of widespread phagocytic action. In one case, neuroma formation was observed close to the proximal end of the cuff and sections through the neuroma showed axons grouped in clusters with their myelin sheaths irregular and thin or completely missing. The observations are much the same as those reported by Ranson (1906), some 70 years ago, and I quote:

> "The individual fibers are much decreased in size, the change affecting chiefly the myelin sheaths. Many fibers are entirely devoid of myelin and there is a tendency for them to be grouped in bundles."

CHAPTER 4

ON SOME BEHAVIOURAL STUDIES INVOLVING THE NATURE AND ROLE OF REFLEXES IN LOCOMOTION

To date the majority of investigations dealing with the reflex control of vertebrate locomotion have been confined to the chronic spinal or the more recent mesencephalic preparations. While these studies have made significant contributions to our understanding of mechanisms underlying the locomotory processes, the findings still have to be extrapolated to behaviour of animals under normal, physiological conditions. The methods described in the previous two chapters offered us an opportunity to examine the nature and role of some reflexes in the distal hindlimb of alert, freely-moving cats.

It is well known from the work of Sherrington and others that stimulation of muscle nerves with shocks that activate fibers smaller than group I generally evokes a flexor reflex, the characteristic pattern being a facilitation of the ipsilateral flexor muscles and an inhibition of the ipsilateral extensors. However, Sherrington and his colleagues also demonstrated that the ipsilateral input to the extensors contained an excitatory component as well. In recent years a number of studies have provided further evidence of these classical findings and many cases of ipsilateral extension have been observed. For example, Hagbarth (1952) showed that stimulation of appropriate skin areas facilitates the ipsilateral monosynaptic extensor reflexes. Subsequently, Holmqvist and Lundberg (1961) occasionally observed facilitation of the monosynaptic reflex in the nerve to

gastrocnemius by stimulation of high threshold afferents from several muscle nerves. Finally, some support for ipsilateral excitatory effects in extensors is available from intracellular studies. Eccles and Lundberg (1959) have observed excitatory post-synaptic potentials (EPSP's) in extensor motoneurons following stimulation of high threshold fibers in ipsilateral muscle nerves. Wilson and Kato (1965) have also observed such effects by selectively stimulating group II afferent fibers in cutaneous and muscle nerves. All of these findings appear to fit with the original postulate of Creed *et al.* (1932) that: ".....in most stimulations of afferent nerves where the reflex rule of excitation of ipsilateral flexors and inhibition of ipsilateral extensors appears to hold, there are obscured contrary effects. It is the existence of these effects which enables any particular level of higher centers or any particular state of the co-ordinating mechanisms to 'set' the lower centers in such a way as to constitute a 'neural balance'."

The present experiments provide a study of one of these effects. We have investigated the effects of single shocks in the tibial and the common peroneal nerves of the EMG activity of the ipsilateral extensors (triceps surae). The results will show that the activation of group II and cutaneous afferents leads to excitation in the ankle extensor muscles. Furthermore, that this excitatory component of the reflex activity is characterized by an early and a late response. The nature of these responses is explained in light of similar findings by Pompeiano (1968), Lund and Pompeiano (1970) and Yanagisawa *et al.* (1976). We have also attempted to extend these

investigations to a preliminary study of treadmill locomotion in cats. It is therefore appropriate to briefly review some contemporary ideas on the extent of reflex control in locomotion.

It is possible to evoke stepping of hindlimbs in an acute spinal cat by nociceptive stimulation of the peri-anal reflex (Sherrington, 1910), or by an intravenous injection of the drug di-hydroxyphenilanin (DOPA) (Grillner, 1973). Even in a curarized spinal animal injected with DOPA, it is possible to observe alternating activity of antagonist muscle nerves in the hindlimb (Jankowska *et al.*, 1967a, b). Thus it would seem that the spinal cord by itself is capable of generating stepping at least of the hindlimbs, but there is some evidence to suggest that the periodicity of such movements is considerably dependent on the tonic afferent inflow (Orlovsky and Feldman, 1972).

The idea of an intraspinal program for the generation of stepping was originally proposed by Graham Brown (1914). He supposed that the 'half-centers' of antagonist muscles of the limb were linked by mutually inhibitory, reciprocal connections. When one of the 'half-centers' was active, the other would be inhibited. During the course of its activity the influence of the active 'half-center' would continually diminish, thereby activating the antagonist by disinhibition. The process was deemed to have a cyclic course even in the absence of afferent input. This basic idea of Brown can often be found in contemporary work, where there is much evidence to support it (Lundberg, 1973; Grillner, 1975). Also notable in this regard are the findings of Jankowska *et al.* (1967a, b), who demonstrated that

the reciprocal inhibition is achieved through inhibitory interneurons, which can be activated by DOPA injections.

All these findings have left the pundits of locomotion asking the question: What role, if any, do afferents, particularly those other than group I, play in the regulation of locomotory activity? For as early as 1910, Sherrington observed that denervation of the foot revealed very minor deficits in the walking behaviour of cats and therefore he was led to believe that afferents from the foot were dispensable to locomotion. Recently, Grillner (1975) has suggested that while these footpad reflexes have no indispensable role for stepping, they may serve as reinforcing stimuli and that relevant deficits would be revealed if cats were allowed to walk on unpredictably slippery or rough surfaces. Some attempts along these lines have been made by introducing perturbations into the rhythmic step cycle and these studies have yielded some interesting results. For example, Forssberg *et al.* (1975) have shown that stimulation of the dorsum of the foot during walking results in a phase-dependent reflex reversal. Tactile stimulation applied during the swing phase of the walking cycle enhanced the flexion of the hindlimb whereas a similar stimulus during the stance phase evoked a shorter but more pronounced extension. The findings of Duysens and Pearson (1976), Duysens (1976) from stimulating the pad, the plantar surface of the foot and a number of peripheral cutaneous nerves reveal similar results to those observed by Forssberg *et al.* (1975). Taken together, these observations support the idea that extensor reflexes elicited from the skin of the distal hindlimb do indeed participate in locomotion.

The aforementioned observations led to a series of preliminary experiments in which we have attempted to determine the role of certain extensor reflexes in evoking phase transitions during the locomotory cycle. To this end, we electrically stimulated two different peripheral nerves as a means of perturbing the step cycle and observed the effects on the rhythmicity of motor output in normal, freely-walking cats. The results indicate that there are some striking similarities between the pattern of step cycle changes in the normal and thalamic cats.

A. RECORDING METHODS

Each recording session was preceded by a threshold determination of the stimulated nerve in the animal under anaesthesia. Single cathodal shocks were applied to the peripheral nerves under study and the resultant compound action potential recorded from the cuff around the sciatic nerve. The maximal amplitude of evoked potentials and the threshold stimulus were determined. In addition, the stimulus strength was varied to obtain a response 10% of the maximal value. In our experience the alert animal would rarely tolerate trains of stimuli in excess of 10-15% of the maximum value and consequently the measurement provided us with a useful index on which to base our range of behavioural stimuli.

The alert cats were then recorded from while in the treadmill with the set-up being much the same as in Fig. 5 (Chapter 2). The EMG activity from the ankle extensors was recorded from one of the leads outside the tibial or the peroneal cuffs, which were also used for stimulation purposes. Concomitantly, neural signals were recorded from

the sciatic nerve and where possible also from the stimulated nerves. The parallel channels carrying neural and EMG activity were fed through preamplifiers, appropriately filtered and displayed on an oscilloscope. All data were recorded on a four-channel FM tape recorder.

For the reflex studies, the nerves (tibial and common peroneal) were stimulated with single pulses 0.01 msec in duration, delivered once every 4 sec at progressively increasing stimulus intensities. The stimuli were delivered from an isolation unit and stimulus markers were also recorded on magnetic tape.

To determine the effect of perturbations on the locomotory cycle, the cats were allowed to walk for a food reward and stimuli were applied in short trains at regular intervals of 4 sec or longer. The interval was chosen such that the 80 msec train of 6 pulses was not synchronized within the step cycle but fell at random within the cycle. The intensity of the stimulus was selected so as not to cause the animal unnecessary pain, but at the same time to be adequate to evoke a behavioural response during walking. The experiments were mostly confined to cut tibial and peroneal nerves, although on occasion we did look at the response of the intact nerves.

B. TREATMENT OF DATA

(i) Reflex

In order to quantify the development of the various responses with increasing stimulus intensities, the neural and EMG records were computer averaged. We used the direct response recorded on the sciatic nerve as a measure for the activity of α motor fibers and the

reflex responses were obtained from the EMG records. Both analog signals were fed through a pair of amplifiers with variable gains and the output levels were restricted to ±1 volt to meet the requirements of the computer. The neural signals were led directly to the computer input whereas the EMG was full-wave rectified and appropriately filtered before being fed into the computer. The program AVER (French, 1973) was used to average both analog inputs triggered by the same stimulus pulse. In all, 15 responses were averaged using a sampling interval of 0.3 msec and the process was repeated for different stimulus intensities. In order to eliminate troublesome stimulus artefacts, the program AVER was also used for inserting delays into the records prior to averaging. The output was then displayed and printed. The amplitudes and latencies of the different responses were determined using another computer program, PEAK. The delays introduced by the filter and the program AVER were taken into account in the final measurement of latencies to the various peaks. Finally, the amplitudes of the reflex responses and of the direct motor response were plotted as a function of stimulus intensity expressed in multiples of threshold (T).

(ii) Locomotion

The data from the walking cat were played back and filmed from an oscilloscope at speeds of 2.5 or 5 cm/sec. Alternately, the records were printed out on a Grass polygraph (model 7D) with a time constant of 20 msec. The step cycle duration was defined as the period between the onsets of subsequent EMG bursts from the ipsilateral ankle

extensors. No quantification was carried out in these preliminary studies but a fairly extensive qualitative analysis of the data was performed.

C. RESULTS

(i) Tibial Nerve

Electrical stimulation of the tibial nerve produces a number of reflex responses in the ankle extensors (Fig. 26A). In addition to the direct action potential recorded on both the sciatic and the tibial nerves, at least two responses were evident. It is well established that the early reflex response is due to monosynaptic excitation of α-motoneurons belonging to synergistic muscles. This is also borne out by the latency measurements of this response. In Fig. 26A, the latency of the direct response (D) corresponds to 1.5-2 msec while that of the monosynaptic reflex (MR) corresponds to 9-12 msec. The most interesting feature of the present experiments is the appearance of at least one and on occasion two late reflex action potentials. The first of these two late reflexes can be referred to polysynaptic excitation of the extensor α-motoneurons (PR). We observed this consistent response at a latency of 30-40 msec on many occasions and at various stimulus intensities. Fig. 27 shows the development of this reflex action potential produced by single shocks with progressively increasing stimulus intensities, expressed as multiples of threshold (T) for the direct response. For comparison, the simultaneous development of the direct response (D) is also given in the same plot. The polysynaptic reflex first appears at a stimulus intensity slightly suprathreshold

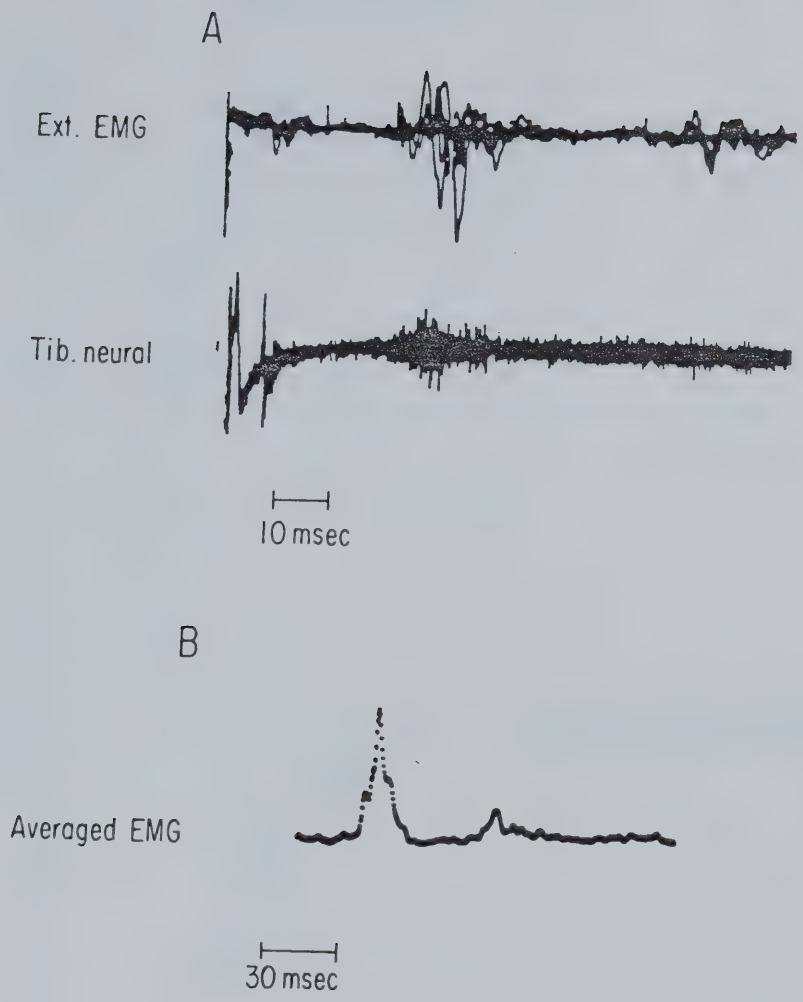


Fig. 26. Nature of direct and reflex responses as a result of stimulating the cut tibial nerve. A) upper trace, shows the EMG reflex responses. Monosynaptic, polysynaptic and a 'late' component are evident; lower trace, neural activity in the tibial nerve shows the direct response as well as some reflex components. B) Computer averaged EMG, which was full-wave rectified and filtered prior to averaging. The polysynaptic and the late reflex waves are quite clear now with latencies of 33.3 and 81.9 msec respectively.

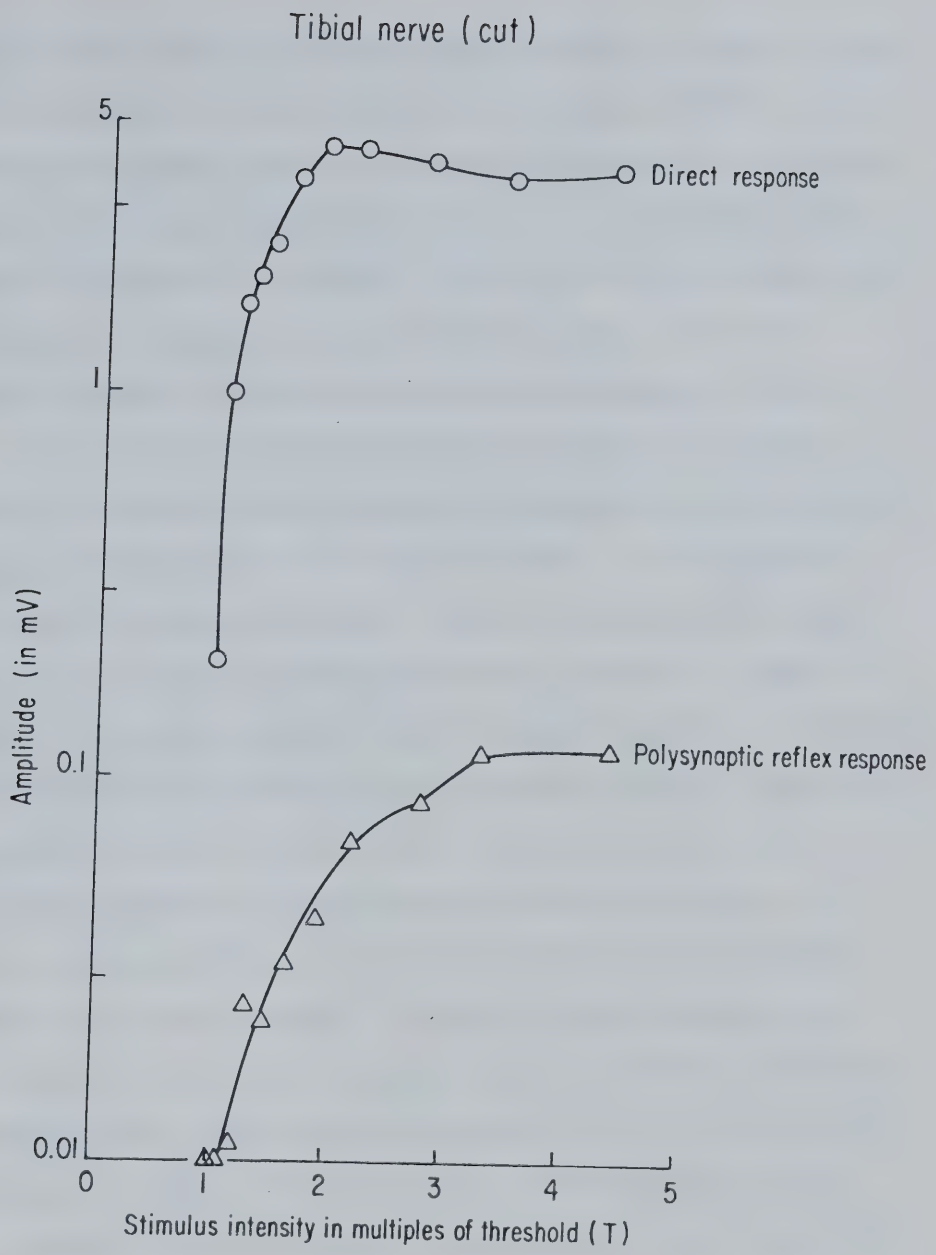


Fig. 27. Development of the direct and reflex responses by stimulating the cut tibial nerve at increasing stimulus intensities. The direct response was measured from the sciatic nerve and the reflex wave from the EMG (extensor) recorded outside the tibial cuff. Note the saturation of the direct response well before the polysynaptic reflex response.

for the direct response and shows a steady increase in amplitude with higher intensities. An important point to be made from this plot is that while the direct response has reached a maximum at an intensity of 1.9 *T*, the reflex response shows no such sign of saturating but continues to increase in amplitude. This would strongly suggest the involvement of cutaneous and high threshold (group II and III) muscular afferents in the mediation of this polysynaptic reflex.

In addition to this polysynaptic reflex, the averaged records did on some occasions reveal the presence of a response with a latency in the order of 75-85 msec (Fig. 26B). This response is almost certainly reflex in nature, since its latency is below that expected for the onset of voluntary activity. Recently, a late extensor reflex with a similar latency has been observed by stimulating the tibial nerve in a thalamic cat (J. Duysens, personal communication). Whilst the preliminary status of these findings precludes any definitive statements as to the nature of this reflex, it is interesting to speculate on the possible pathways responsible for its rather long reflex pathway. A number of spinal pathways have been shown to be involved in extensor and flexor reflexes. Of these the propriospinal and the spino-bulbospinal (SBS) are the ones most thoroughly investigated (Shimamura *et al.*, 1965; Jankowska *et al.*, 1973). However, the SBS pathway is excitatory to flexor motoneurons only (Shimamura *et al.*, 1965) and consequently its implication may be ruled out in the present case. The involvement of higher centers cannot be denied, nor can we exclude some form of local autogenic excitation of the extensors from the initial polysynaptic reflex.

Let us now turn our attention to the results of stimulating the tibial nerve during locomotion. When the stimuli fell within the stance phase of the ipsilateral hindlimb, a marked effect on the ipsilateral extensor activity was observed (Fig. 28A). Both amplitude and duration of the EMG burst were increased. Stimulation of the nerve during the last part of the flexion period induced a premature extension onset (Fig. 28B) and in some cases this ipsilateral extensor activity was prolonged to cause a step cycle prolongation (Fig. 28C). These premature step cycle transitions occurred only when the stimulus was delivered during a certain period of the cycle. Unfortunately, no reliable method was available to quantify these effects. However, the findings are in accord with those of Forssberg *et al.* (1975) and Duysens (1976), who showed similar phase-dependent reflex reversals in chronic and thalamic cats, respectively. An interesting feature evident from Fig. 28 is the presence of a response in the EMG 32 msec after the onset of the stimulus train. This is probably the polysynaptic reflex response obtained earlier by graded stimulation of the tibial nerve in the alert cat.

(ii) Common Peroneal Nerve

Stimulation of the cut ipsilateral peroneal nerve evoked excitatory responses in the ankle extensors. Fig. 29 shows the two-component reflex excitation, the latencies to the first and second of these responses being 11.4-14.4 and 28.5-31.5 msec, respectively. That the observed EMG pickup is merely the result of autogenic excitation of the ankle flexors can be ruled out on the basis that the common peroneal

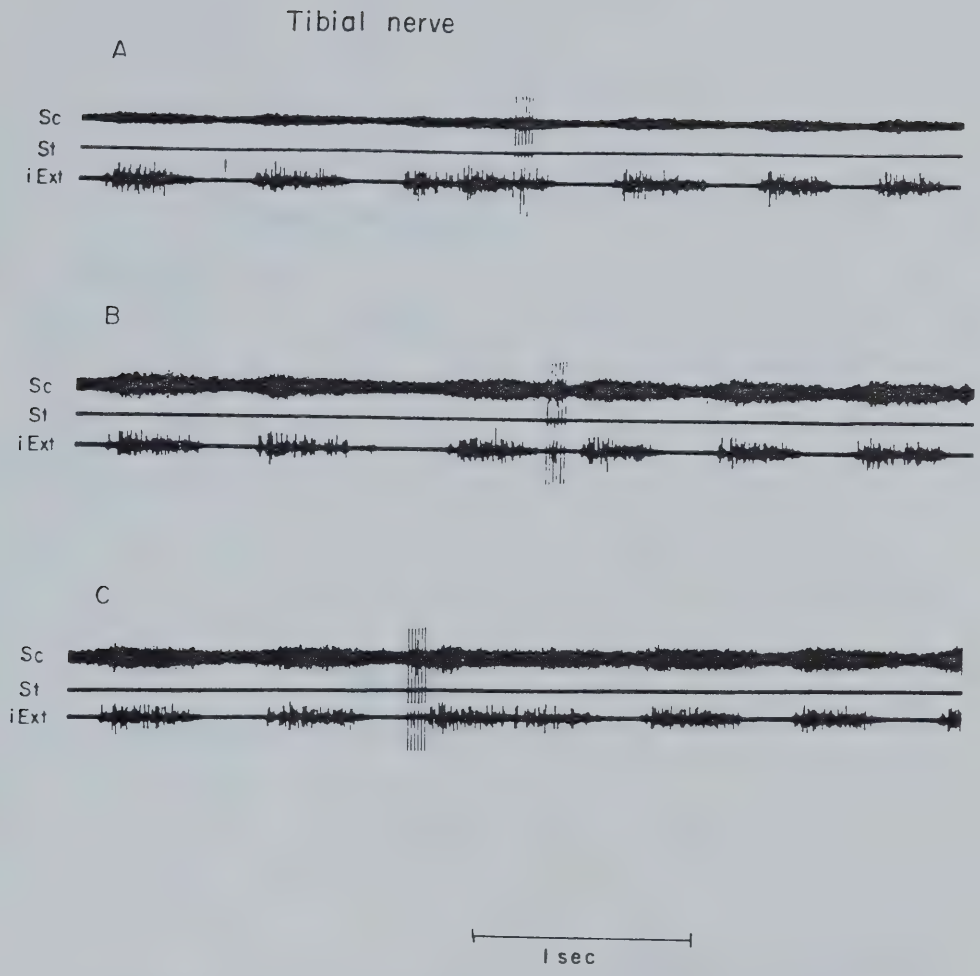


Fig. 28. Effect of tetanic tibial nerve stimulation on the ipsilateral extensor activity during the step cycle of a normal cat walking on the treadmill. Stimulation (St) during the extension or stance phase prolongs the intensity and duration of the ipsilateral extensor (iExt) burst. When given during the swing phase, a premature onset of the extensor burst is evident (B). This phase reversal results in the shortening of the step cycle. (Sc) denotes activity in the sciatic nerve.

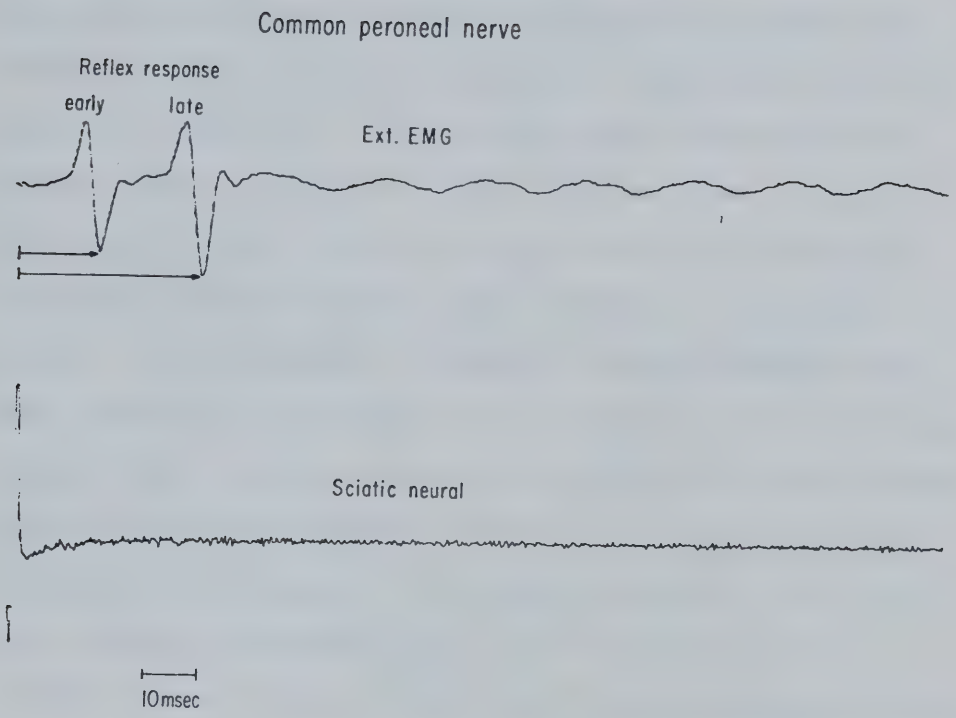


Fig. 29. Computer averaged direct and reflex responses as a result of stimulating the cut common peroneal nerve at twice the threshold stimulus intensity (2.0 x T). The latencies to the early and late reflex waves (indicated by arrows) are 14.4 and 31.5 msec, respectively. The direct wave was measured off the neural trace from the sciatic nerve, the latency for this being 1.5 to 2 msec.

nerve was cut proximal to these flexors which it innervates. Graded stimulation of the nerve produced an increase in both of the reflex responses as well as the direct action potential, which was again recorded on the sciatic nerve (Fig. 30). However, the pattern of this progressive increase for the two reflexes shows considerable similarity to that observed by tibial nerve stimulation. The early reflex first appears at 1.33 x T and the later response makes its presence obvious at 1.48 x T. In any case, the direct response is well saturated by this time and this again implies the involvement of cutaneous and high threshold muscle afferents in the reflex activity. The very presence of extensor reflexes evoked by flexor nerve stimulation is surprising, since inhibitory connections between antagonist muscle groups have been well established for some time now. However, the excitatory action of flexor nerve stimulation has been observed in a few instances. For example, Creed *et al.* (1932) did, when stimulating the peroneal nerve, see the appearance of an excitatory reflex followed by inhibition in the gastrocnemius muscle. More recently, Yanagisawa *et al.* (1976) have provided some evidence for a facilitatory effect on the triceps surae H-reflex by peroneal nerve stimulation.

A train of stimuli delivered during the stance phase of the step cycle evoked an increase in ipsilateral EMG activity (Fig. 31A, B and C). In some instances the EMG burst was quite prolonged, but we have no evidence as yet of phase-dependent reflex reversals. Meanwhile, Duysens (1976), in the thalamic cat, has demonstrated a suppression of the ipsilateral extensors when stimulating the peroneal nerve under

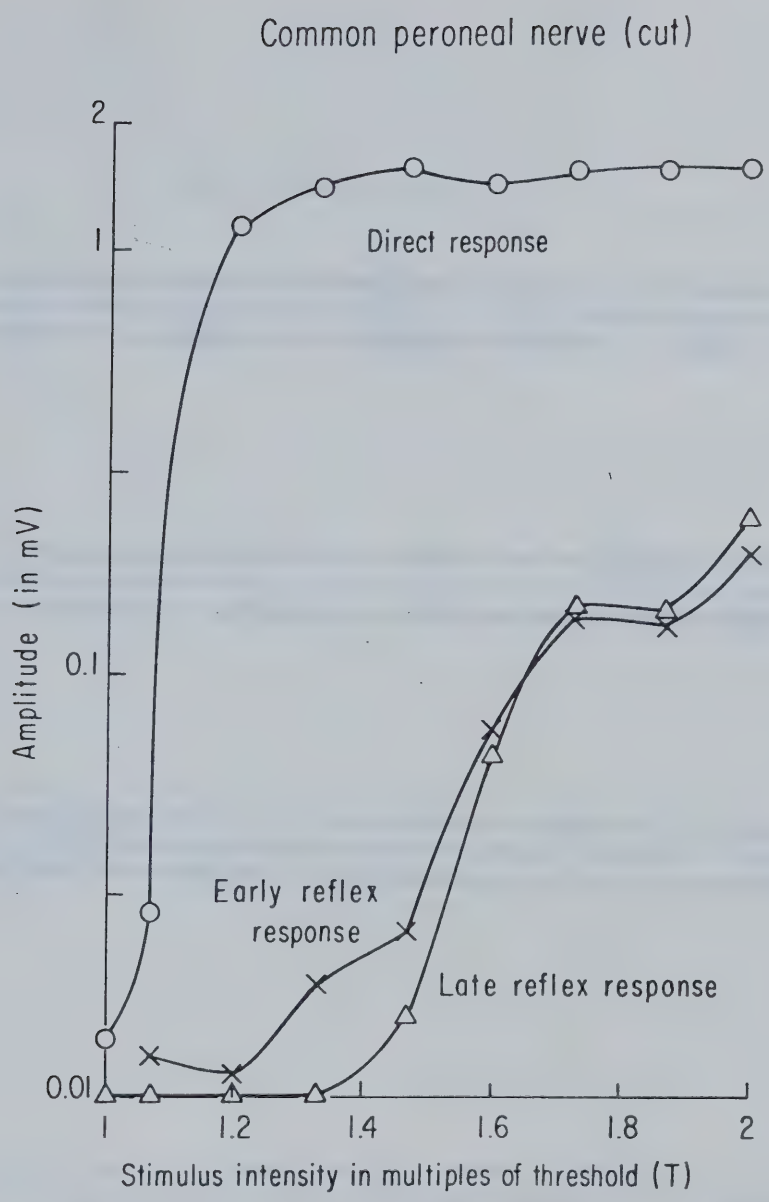


Fig. 30. Development of direct and reflex responses by stimulating the cut common peroneal nerve at increasing stimulus intensities. The reflex responses were measured from the averaged EMG and the direct response from the sciatic nerve. Note the saturation of the direct response well in advance of the reflex responses which continue to get larger.

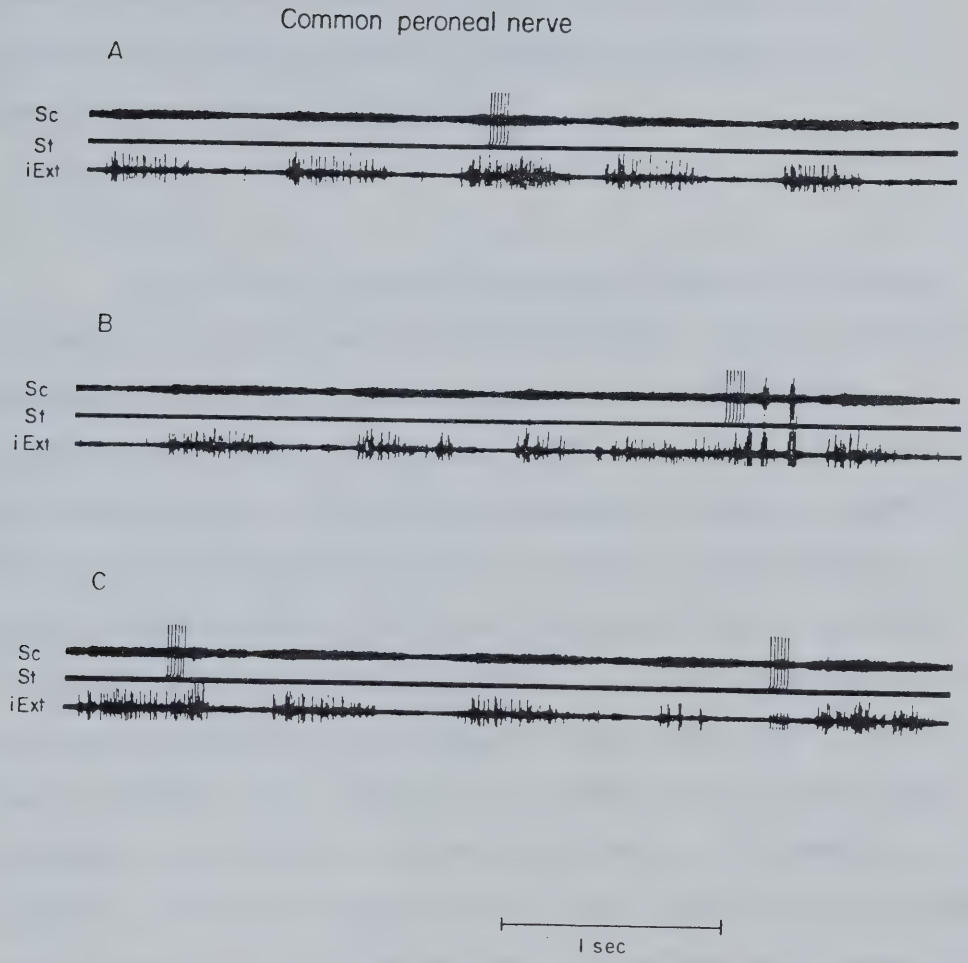


Fig. 31. Effect of tetanic common peroneal nerve stimulation on the ipsilateral extensor activity during locomotion of a normal cat. A short train of six electrical shocks (St) delivered during the extension or stance phase evokes a prolonged and intense discharge in the ipsilateral ankle extensors (A, B and C). Activity in the sciatic nerve (Sc) is also shown.

similar conditions. His observations are in line with the current notions about the role of cutaneous afferents (or more generally termed Flexor Reflex Afferents [FRA's]) in the 'flexor reflex' response, this response being facilitatory to the flexor and inhibitory to extensor motoneurons.

D. DISCUSSION

Over the past 10 years a considerable amount of evidence has been put forth to suggest that the locomotory rhythm is centrally generated and modified by sensory input from various receptors (Grillner, 1975; Pearson and Duysens, 1976). It is further proposed that the swing phase of this cyclic movement is rigidly and centrally programmed whereas the stance phase is subject to modification by ongoing sensory feedback. The present data support this hypothesis. A prolongation of the extension phase due to afferent stimulation is a consistent observation, but no change of the flexion phase is apparent from our records. This type of a reinforcing effect on the extensor motoneurons has important functional consequences. For example, such reinforcing reflexes can compensate for sudden, unexpected load changes during locomotion. In the mesencephalic cat walking on a treadmill, resistance of the leg during the stance phase evokes a rather marked increase in EMG recorded from the leg extensors (Severin, 1970). The sensory input responsible for producing this type of an effect is as yet unidentified, but muscle spindles in the leg extensors have been implicated. Recent work suggests that stimulation of various skin areas of the foot during the stance phase also increases extensor EMG

(Forssberg *et al.*, 1975; Duysens and Pearson, 1976). Thus, the reflexes arising from activity in cutaneous afferents in the foot also serve to reinforce activity in extensor motoneurons during the stance phase of leg movement. The behaviour observed in a normal, freely-walking cat would support such a role for cutaneous and high threshold muscle afferents.

Lundberg (1973) has suggested the existence of excitatory as well as inhibitory pathways from the so-called 'flexor reflex afferents' to ipsilateral extensor motoneurons. Our results, together with the occasional findings of others, demonstrate that both group II and III as well as the cutaneous afferents produce effects which are not at all typical of the flexor reflex pattern. Therefore, it would appear that the term 'flexor reflex afferents' which has been used to include such high threshold afferents is unjustified and should be discarded.

Finally, although the present findings are for the most part complimentary to those observed in the chronic spinal or the thalamic animal, there are some inconsistencies also. It is these inconsistencies which underline the dangers of extrapolating results from 'unnatural' preparations to those observed under normal physiological conditions.

CHAPTER 5

GENERAL DISCUSSION

In Chapter 1, I alluded to the use of chronic nerve stimulation in the study of pain fibers as early as 1933 (Cannon, 1933). More recently, chronic stimulation of mammalian nerves, nerve tracts and even areas of the brain have been used in a variety of clinical situations. For example, some success has been achieved in stimulating the phrenic nerve to assist respiration and in stimulating the dorsal columns or the cerebellum to relieve intractable pain in patients. The methods described here would also be of considerable use in improving the success of the aforementioned clinical aims. However, any discussion of the methodology described here would be incomplete without reference to its relevance to prosthetics.

In spite of recent developments in powered prosthetic components, by far the majority of above-elbow prostheses in use today are powered by the motion of the shoulder girdle and residual limb. The disadvantages of such cable-controlled arms are from harness discomfort and also the necessity to generate fairly large forces and excursions. The development of a myoelectric device has to some extent tried to cope with these difficulties. In its simplest form, such an above-elbow prosthesis is powered by surface electromyographic signals that are picked up over the remnant but dysfunctional biceps and triceps muscles. The EMG signals are electronically processed, combined as a biological antagonist-agonist pair and fed into an electromechanical type of a servo, which controls the elbow force and angle. However,

EMG controlled limbs have some inherent drawbacks. Chief amongst these is the lack of available control sites for greater degrees of freedom. A considerable number of amputees lack sufficient stumps of muscle to generate control signals for the artificial limb, and even when such signals are available, it is only possible to control the elbow joint. For performing wrist or hand movements, one must contemplate an alternate source of command signals.

The possibility for more control sites is provided by the severed nerves of an amputee. The technique outlined in this thesis make it feasible to sample the neural traffic in peripheral nerves, which are after all the conduit for muscular control from the spinal cord and higher centers. As mentioned earlier, by stimulating the peripheral nerve, it is also possible to provide information analogous to that generated by the distal sensory end organs. The fate of voluntary activity in severed nerves is therefore a crucial question to answer. The results presented here suggest that a severed nerve deprived of its target organ is incapable of sustaining voluntary signals for any appreciable length of time. What is more promising is the fact that these cut nerves do remain electrically excitable for periods up to 6 months. The practice of cross-suturing nerves to restore normal function has met with limited success in a number of higher vertebrates and even man (Mark, 1969). If this procedure was extended say to cross-suturing a more distal nerve to more proximal musculature, there is a possibility that voluntary activity may be recordable more or less indefinitely. There is considerable evidence in some recent studies to support such a hypothesis (Cull, 1974;

Mendell *et al.*, 1975). By diverting the efferent flow of neural signals for which no relevant musculature exists, one could conceivably provide it with a target organ. This kind of a procedure would then offer a choice of EMG or neural signals for control purposes. In such a situation there is much to be said for using either of these natural signals in activating prostheses. EMG signals, because of their relatively larger magnitude, are preferable. However, the methods outlined in Chapter 3 show that neural amplitudes can to a certain degree be increased by the use of longer cuffs. Moreover, it is possible, by using cuffs, to record from both central and peripheral fibers without too much attenuation (R.B. Stein, personal communication). The EMG pickup on the other hand is restricted territorially. Finally, the optimization procedures developed here would be rather ineffective for the relatively low frequency electromyographic signals.

Perhaps a more immediate application of the methods described here is to behavioural studies of the type described in Chapter 4. As was pointed out earlier, the central control of the basic locomotory rhythm is a fairly well accepted fact but the extent to which peripheral feedback modifies such central patterns is still a contentious issue. Also unclear is the role of some bifunctional muscles in reflex regulation of locomotion (Perret *et al.*, 1975). These and other related questions can only be properly resolved by tests on animals performing under normal physiological conditions. The improvements in cross-correlation techniques should be particularly useful in the study of motor systems where functions during posture and locomotion of various, clearly defined afferent and efferent fibers are still

controversial (Stein *et al.*, 1973).

Finally, the application of chronic neural recording need not and should not be confined to the peripheral nervous system. Recent attempts to record sympathetic nerve activity in freely-moving, conscious animals have yielded some interesting results. For example, Schad and Seller (1975) have investigated the effects of various pharmacological agents on the activity in the renal nerve of cats with intact and denervated baroreceptors. As well, they have observed changes in the pattern of neural activity in various states of anaesthesia and consciousness. Such studies would give considerable insight into the regulation of the autonomic nervous system during various behavioural states.

Recently, the principle of the recording techniques described here and elsewhere have also been applied to the study of normal and dystrophic mice (J.A. Hoffer, personal communication) in the hope of elucidating the relative contributions of the nerve, the muscle and other systemic factors. In an era where solutions to various pathological disorders of the nervous system increasingly call upon an assortment of clinical and basic knowledge, we hope that the methods presented here will make their due contribution.

BIBLIOGRAPHY

Acheson, G.H., Lee, E.S. and Morison, R.S. (1942) A deficiency in the phrenic respiratory discharges parallel to retrograde degeneration. J. Neurophysiol. 5: 269-273.

Bak, M.J., Loeb, G.E., Schmidt, E.M. and Salcman, M. (1975) Parylene-C as a chronically stable, reliable dielectric coating for implanted electrodes. Soc. Neurosci. Abstr. 5: 1124.

Barr, M.L. and Hamilton, J.D. (1948) A quantitative study of certain morphological changes in spinal motor neurones during axon reaction. J. Comp. Neurol. 89: 93.

Bockris, J. and Reddy, A. (1970) Modern Electrochemistry. Plenum Press, New York.

Boyd, I.A. and Davey, M.R. (1968) Composition of Peripheral Nerves. E & S Livingstone Ltd., Edinburgh.

Brindley, G.S. (1972) Electrode arrays for making long-lasting electrical connexions to spinal roots. J. Physiol., Lond. 222: 135-136P.

Brown, T.G. (1914) On the nature of the fundamental activity of the nervous system; together with an analysis of the conditioning of rhythmic activity in progression and a theory of the evolution of function in the nervous system. J. Physiol., Lond. 48: 18-46.

Buchthal, F. and Rosenfalck, A. (1966) Evoked action potentials and conduction velocity in human sensory nerves. Brain Res. 3: 1-120.

Cajal, S.R. (1928) Degeneration and Regeneration of the Nervous System. R.M. May (Trans.), Oxford University Press, London.

Cannon, B. (1933) A method of stimulating autonomic nerves in the unanaesthetized cat with observations on the motor and sensory effects. Am. J. Physiol. 105: 366-372.

Cavanaugh, M.W. (1951) Quantitative effects of the peripheral innervation area on nerves and spinal ganglion cells. J. Comp. Neurol. 94: 181.

Cragg, B.G. (1970) What is the signal for chromatolysis? Brain Res. 23: 1-21.

Cragg, B.G. and Thomas, P.K. (1961) Changes in conduction velocity and fibre size proximal to peripheral nerve lesions. J. Physiol., Lond. 157: 315-327,

Creed, R.S., Denny-Brown, D., Eccles, J.C., Liddel, E.G.T. and Sherrington, C.S. (1932) Reflex Activity of the Spinal Cord. Oxford University Press, London.

Cull, R.E. (1974) Role of nerve-muscle contact in maintaining synaptic connections. Exp. Brain Res. 20: 307-310.

Cull, R.E. (1975) Effect of sensory nerve division on the afferent synapses of axotomized motor neurones. Exp. Brain Res. 22: 421-425.

DeLuca, C.J. and Gilmore, L.D. (1976) Voluntary nerve signals from severed mammalian nerves: long-term recordings. Science 191: 193-195.

Diefenderfer, A.J. (1972) Principles of Electronic Instrumentation. W.B. Saunders, Philadelphia.

Duysens, J. (1976) Reflex control of phase transitions in walking of thalamic cats. Submitted for publication.

Duysens, J. and Pearson, K.G. (1976) The role of cutaneous afferents from the distal hindlimb in the regulation of the step cycle of thalamic cats. Exp. Brain Res. 24: 245-255.

Eccles, R.M. and Lundberg, A. (1959) Synaptic action on motoneurons by afferents which may evoke the flexion reflex. Archs. ital. Biol. 97: 199-221.

Edds, M.V. Jr. (1945) Prevention of nerve regeneration and neuroma formation by caps of synthetic resin. J. Neurosurg. 2: 507.

Evarts, E.V. (1966) Pyramidal tract activity associated with a conditioned hand movement in the monkey. J. Neurophysiol. 29: 1011-1027.

Forssberg, G.H., Grillner, S. and Rossignol, S. (1975) Phase dependent reflex reversal during walking in chronic spinal cats. Brain Res. 85: 103-107.

Frank, K. (1968) Some approaches to the technical problem of chronic excitation of peripheral nerves. Ann. Otology, Rhinology and Laryngology 77: 761-772.

French, A.S. (1973) Averaging of neurophysiological data prior to frequency domain analysis. Comp. Prog. Biomed. 3: 113-122.

French, A.S. and Holden, A.V. (1971) Frequency domain analysis of neurophysiological data. Comput. Prog. Biomed. 1: 219-234.

Grafstein, B. (1975) The nerve cell body response to axotomy. Exp. Neurol. 48: 32-51.

Grillner, S. (1973) Locomotion in the spinal cat. In: Control of Posture and Locomotion, ed. by R.B. Stein, K.G. Pearson, R.S. Smith and J.B. Redford. Plenum Press, New York. p. 515-535.

Grillner, S. (1975) Locomotion in vertebrates: central mechanisms and reflex interaction. Physiol. Rev. 55: 247-304.

Guth, L. (1956) Regeneration in the mammalian peripheral nervous system. Physiol. Rev. 36: 441-478.

Hagbarth, K.-E. (1952) Excitatory and inhibitory skin areas for flexor and extensor motoneurones. Acta physiol. scand. 26, suppl. 94.

Heetberks, W.J. and Williams, W.J. (1975) Partition of gross peripheral nerve activity into single unit responses by correlation techniques. Science 188: 373-375.

Hoffer, J.-A. (1975) Long-term peripheral nerve activity during behaviour in the rabbit: the control of locomotion. Unpublished Ph.D. Thesis, Department of Biophysics, Johns Hopkins University, Baltimore, Maryland.

Hoffer, J.-A., Marks, W.B. and Rymer, W.Z. (1974) Nerve fiber activity during normal movements. Soc. Neurosci. Abstr. 4: 300.

Holmqvist, B. and Lundberg, A. (1961) Differential supraspinal control of synaptic actions evoked by volleys in the flexion reflex afferents in alpha motoneurones. Acta physiol. scand. 54: suppl. 186.

Hubel, D.H. (1957) Single unit activity in visual cortex of the unanaesthetized cat. Fed. Proc. 16: 63.

Hydén, H. (1943) Protein metabolism in the nerve cell during growth and function. Acta physiol. scand., suppl. 17.

Jacobson, M. (1970) Developmental Neurobiology. Holt, Rinehart & Winston, New York.

Jankowska, E., Jukes, M.G.M., Lund, S. and Lundberg, A. (1967a) The effect of DOPA on the spinal cord. 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors. Acta physiol. scand. 70: 369-388.

Jankowska, E., Jukes, M.G.M., Lund, S. and Lundberg, A. (1967b) The effect of DOPA on the spinal cord. 6. Half-centre organization of interneurones transmitting effects from the flexor reflex afferents. Acta physiol. scand. 70: 389-402.

Jankowska, E., Lundberg, A. and Stuart, D. (1973) Propriospinal control of last-order interneurones of spinal reflex pathways in the cat. Brain Res. 53: 227-231.

Lund, S. and Pompeiano, O. (1970) Electrically induced monosynaptic and polysynaptic reflexes involving the same motoneuronal pool in the unrestrained cat. Archs. ital. Biol. 108: 130-153.

Lundberg, A. (1973) The significance of segmental spinal mechanisms in motor control. Proc. Symp. Intern. Biophys. Congr., 4th, Moscow.

Mann, R.W. (1973) Prosthesis control and feedback via noninvasive skin and invasive peripheral nerve techniques. In: Neural Organization and Its Relevance to Prosthetics. Symposia Specialists, Miami. pp. 177-195.

Mannard, A., Stein, R.B. and Charles, D. (1974) Regeneration electrode units: implants for recording from single peripheral nerve fibers in freely moving animals. Science 183: 547-549.

Marinesco, G. (1892) Ueber Veränderungen der Nerven und des Rückenmarkes nach Amputationen; ein Beitrag zur Nerventrophik. Neurol. Zentbl. 11: 463-467,

Mark, R.F. (1969) Matching muscles and motoneurones. A review of some experiments on motor nerve regeneration. Brain Res. 14: 245-254.

Mendell, L.M., Munson, J.B. and Scott, J.G. (1974) Connectivity changes of Ia afferents on axotomized motoneurones. Brain Res. 73: 338-342.

Mendell, L.M. and Scott, J.G. (1975) The effect of peripheral nerve cross-union on connections of single Ia fibers to motoneurons. Exp. Brain Res. 22: 221-234.

Mendell, L.M., Munson, J.B. and Scott, J.G. (1976) Alterations of synapses on axotomized motoneurones. J. Physiol., Lond. 255: 67-79.

Orlovsky, G.N. and Feldman, A.G. (1972) On the role of afferent activity in generation of stepping movements. Neurophysiol. 4: 401-409 (Engl. trans., p. 311-317).

Pearson, K.G. and Duysens, J. (1976) Function of segmental reflexes in the control of stepping of cockroaches and cats. In: Neural Control of Locomotion, ed. by R.E. Herman, S. Grillner, D. Stuart, P. Stein. Plenum Press, New York, in press.

Perret, C. and Cabelguen, J.M. (1975) A new classification of flexor and extensor muscles revealed by study of the central locomotor program in the deafferented cat. Exp. Brain Res. 23: 160.

Pollak, V. (1974a) An equivalent diagram for the interface impedance of metal needle electrodes. Med. Biol. Engng. 12: 454-459.

Pollak, V. (1974b) Computation of the impedance characteristic of metal electrodes for biological investigations. Med. Biol. Engng. 12: 460-464.

Pollak, V. (1974c) Impedance measurements on metal needle electrodes. Med. Biol. Engng. 12: 606-612.

Pompeiano, O. (1968) Monosynaptic and polysynaptic reflex excitation of motoneurones to plantar muscles in the unrestrained cat. Brain Res. 10: 252-256.

Poth, E.J. and Fernandez, E.B. (1944) Prevention of neuroma formation by encasement of the severed nerve end in rigid tubes. Proc. Soc. Exp. Biol. Med. 56: 7.

Purves, D. (1975) Functional and structural changes in mammalian sympathetic neurones following interruption of their axons. J. Physiol., Lond. 252: 429-463.

Ranson, S.W. (1906) Retrograde degeneration in the spinal nerves. J. Comp. Neurol. 16: 265-293.

Roberts, W.M. and Hartline, D.K. (1975) Separation of multi-unit nerve impulse trains by a mutli-channel filter algorithm. Brain Res. 94: 141-149.

Robinson, D.A. (1968) The electrical properties of metal microelectrodes. Proc. I.E.E.E. 56: 1065-1071.

Ruch, T.C. and Patton, H.D. (1965) Physiology and Biophysics. W.B. Saunders, Philadelphia and London. pp. 65-66.

Schad, H. and Seller, H. (1975) A method for recording autonomic nerve activity in unanaesthetized, freely moving cats. Brain Res. 100: 425-430.

Schmidt, E.M., Bak, M.J., McIntosh, J.S. and Stevenson Thomas, J. (1975) Control of operantly conditioned firing patterns of single precentral cortical cells. Soc. Neurosci. Abstr. 1: 177.

Severin, F.V. (1970) On the role of γ-motor system for extensor α-motoneurone activation during controlled locomotion. Biofizika 15: 1096-1102.

Sherrington, C.S. (1910) Flexion-reflex of the limb, crossed extension reflex, and reflex stepping and standing. J. Physiol., Lond. 40: 28-121.

Shimamura, M., Mori, S. and Yamauchi, T. (1965) Effects of spino-bulbospinal reflex volleys on extension motoneurons of the hindlimb in cats. J. Neurophysiol. 30: 319-332.

Skilling, H.H. (1957) Electrical Engineering Circuits. John Wiley & Sons, Inc., New York.

Stein, R.B. and Pearson, K.G. (1971) Predicted amplitude and form of extracellularly recorded action potentials from unmyelinated fibres. J. Theor. Biol. 32: 539-558.

Stein, R.B., Pearson, K.G., Smith, R.S. and Redford, J.B., editors (1973) Control of Posture and Locomotion. Plenum Press, New York.

Stein, R.B., Charles, D., Davis, L., Jhamandas, J. and Nichols, T.R. (1975) A method for chronic recording from nerve fibers in experimental animals and man. Can. Fed. Biol. Soc. 18: 39.

Stein, R.B., Charles, D., Davis, L., Jhamandas, J., Mannard, A. and Nichols, T.R. (1975) Principles underlying new methods for chronic neural recording. Can. J. Neurol. Sci. 2: 235-244.

Stein, R.B., Nichols, T.R., Jhamandas, J., Davis, L. and Charles, D. (1976) Stable long-term recordings from cat peripheral nerves. Submitted for publication.

Stein, R.B., Jhamandas, J. and Nichols, T.R. (1976) Long-term recording of electrical activity of mammalian nerves following axotomy. Soc. Neurosci. Abstr. 6, in press.

Strain, R.E. Jr. and Olson, W.H. (1975) Selective damage of large diameter peripheral nerve fibers by compression: an application of Laplace's Law. Exp. Neurol. 47: 68-80.

Tasaki, I. (1964) A new measurement of action currents developed by single nodes of Ranvier. J. Neurophysiol. 27: 1199-1206.

Vallbo, Å.B. (1971) Muscle spindle response at the onset of isometric voluntary contractions in man. Time difference between fusimotor and skeletomotor effects. J. Physiol., Lond. 218: 405-431.

Warrington, W.B. (1899) Further observations on the structural alterations observed in nerve cells. J. Physiol., Lond. 24: 464.

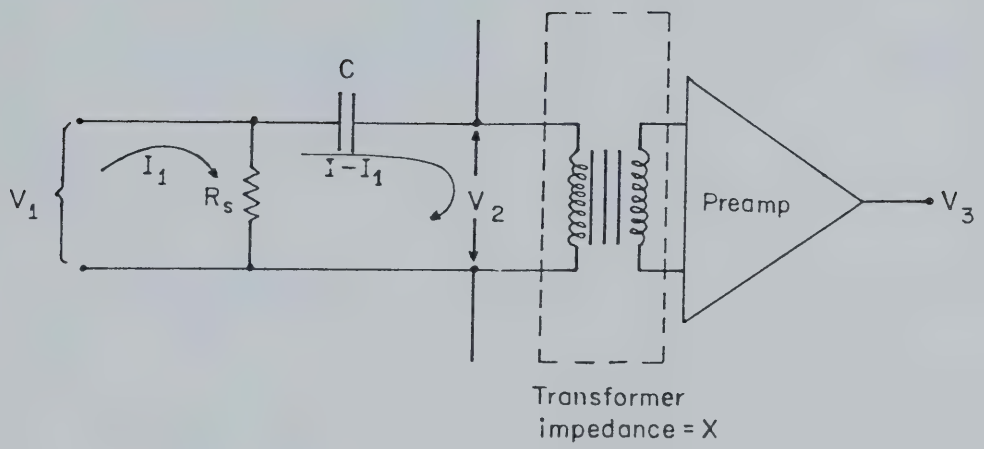
Watson, W.E. (1965) An autoradiographic study of the incorporation of nucleic acid precursors by neurones and glia during nerve regeneration. J. Physiol., Lond. 180: 741.

Wilson, V.J. and Kato, M. (1965) Excitation of extensor motoneurons by group II afferent fibers in ipsilateral muscle nerves. J. Neurophysiol. 28: 545-554.

Yanagisawa, N., Tanaka, R. and Tsukagoshi, H. (1976) Reciprocal facilitation of the triceps surae motoneurons by Ib Golgi afferents of the peroneal nerve in man. Int. Symp. on Human Reflexes and Motor Disorders. Brussels, Belgium/ p. 174.

APPENDIX I

Current Source Analysis



$$I_1 R_s = V_1 \qquad \text{Eqn. 1}$$

$$(I - I_1)\left[X + \frac{1}{(j\omega c)^n}\right] = V_1 \qquad \text{Eqn. 2}$$

$$(I - I_1)X = V_2 \qquad \text{Eqn. 3}$$

Substitute Eqn. 1 in Eqn. 2 to obtain

$$(I - I_1)\left[X + \frac{1}{(j\omega c)^n}\right] = I_1 R_s$$

Let $M = \left[X + \frac{1}{(j\omega c)^n}\right]$

$$\therefore \qquad IM = (M + R_s)I_1$$

$$\therefore \qquad I_1 = \frac{IM}{(M + R_s)} \qquad \text{Eqn. 4}$$

Substitute Eqn. 4 in Eqn. 3 to obtain

$$V_2 = I\ [1 - \frac{M}{M + R_s}]\ X \qquad \text{Eqn. 5}$$

From Eqn. 1,

$$V_1 = \frac{IM}{(\frac{M}{R_s} + 1)} \qquad \text{Eqn. 6}$$

$$\frac{V_3}{V_1} = \frac{V_2}{V_1} \times \frac{V_3}{V_2} \qquad \text{Eqn. 7}$$

From Eqn. 6

$$I = V_1\ [\frac{\frac{M}{R_s} + 1}{M}] = V_1\ (\frac{1}{R_s} + \frac{1}{M}) \qquad \text{Eqn. 8}$$

To measure amplitude ratio, we must obtain the ratio $\frac{V_3}{I}$.

$\therefore$ Using Eqn. 8

$$\frac{V_3}{V_1} \times \frac{V_1}{I} = \frac{V_3}{V_1}\ \ (\frac{1}{\frac{1}{R_s} + \frac{1}{M}}) = \frac{V_3}{V_1}\ (\frac{R_s M}{M + R_s})$$

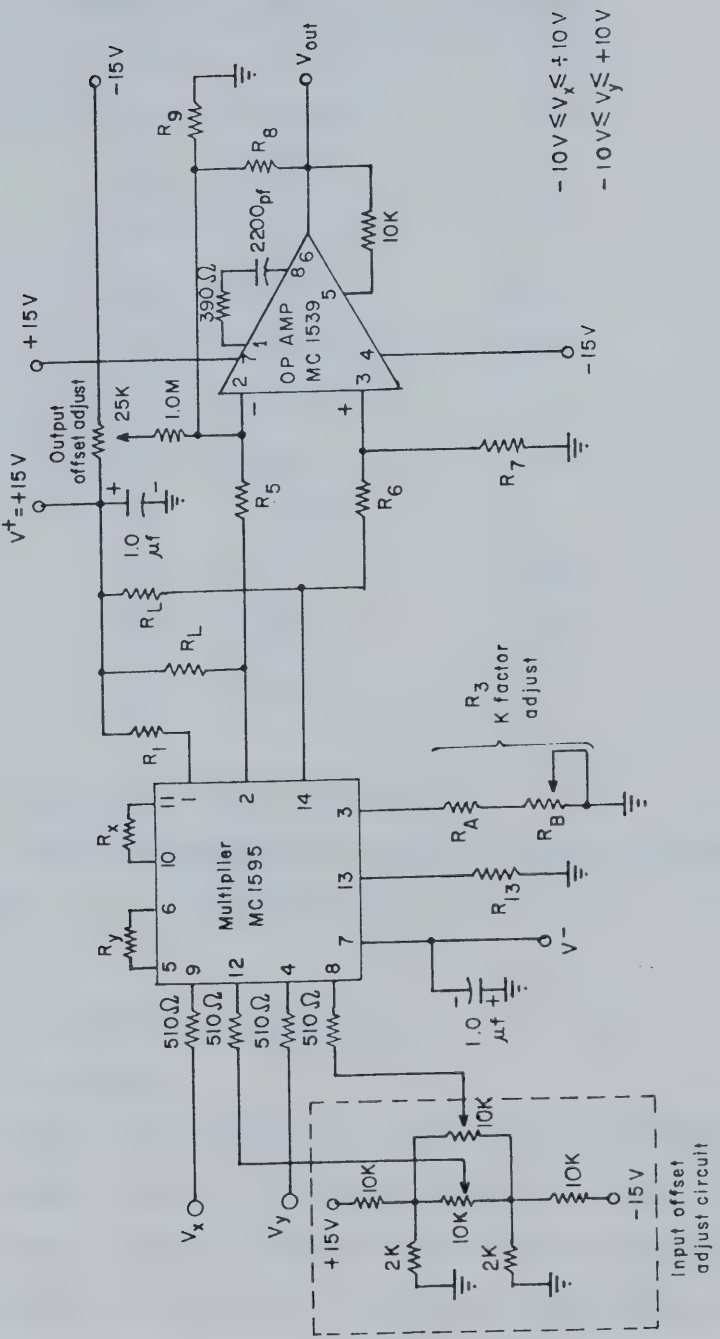
$$\therefore \qquad \frac{V_3}{I} = \frac{V_3}{V_1}\ (\frac{R_s M}{M + R_s})$$

where R_s is known, $|M| = \sqrt{(X)^2 + \frac{1}{(j\omega c)^{2n}}}$ and $\frac{V_3}{V_1}$ can be calculated using Eqns. 5, 6 and 7 together with measured values of $\frac{V_3}{V_2}$ (using a voltmeter).

Small computer programs (in FOCAL) were used to perform the mathematical operations and a value for amplitude ratio (V_3/IR_s) calculated at each frequency (see Fig. 8, Chapter 2).

APPENDIX II

Analog multiplier with OP-AMP level shift,- circuit diagram



Resistor	(Unit)	Tolerance
R_1	= 1.2 KΩ	5%
R_5	= 121 KΩ	1%
R_6	= 100 KΩ	1%
R_7	= 11 KΩ	1%
R_8	= 910 KΩ	1%
R_9	= 13.7 KΩ	1%
R_{13}	= 13.7 KΩ	1%
R_A	= 12 KΩ	5%
R_B	= 5.0 KΩ	20%
R_L	= 12 KΩ	0.5%
R_X	= 15 KΩ	5%
R_Y	= 15 KΩ	5%

Static Error and Scale Factor Adjustment

To obtain useable output accuracy, several adjustments must be made in the external multiplier circuitry.

Procedure (AC Voltmeter or Oscilloscope)

A X-Y Offset Adjust

1. Connect an AC voltmeter or oscilloscope to the output
2. Connect 1.0 KHz, 1.0 V p-p oscillator to Y input, ground X input, adjust X offset (from Pin 12) for an output null
3. Connect 1.0 KHz, 1.0 V p-p oscillator to X input, ground Y input, adjust Y offset (from Pin 8) for an output null

B Output Offset Adjust

1. For circuit shown, adjust "output offset adjust" potentiometer for zero output

C Scale Factor Adjust

1. Set V_X = +5.0 V DC, V_Y = +5.0 V DC and adjust gain potentiometer (K factor) for +2.5 V DC output
2. To check, let V_X = -5.0 V DC, V_Y = -5.0 V DC and check for +2.5 V DC output - if error occurs, repeat steps A, B and C